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ScienceDirect
Journal of Photochemistry and Photobiology B: Biology 85 (2006) 28–38
Journal of Photochemistry and Photobiology B:Biology
www.elsevier.com/locate/jphotobiol

Antibacterial activity of tetraaryl-porphyrin photosensitizers: An in vitro study on Gram negative and Gram positive bacteria

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Received 20 December 2005; received in revised form 19 April 2006; accepted 19 April 2006
Available online 5 June 2006

Abstract

Background: Photodynamic therapy exploits visible light and photosensitizers to inactivate cells and this methodology is currently used for the treatment of several types of malignancy. Although various tumours are successfully treated with PSs and light, the application on microorganisms (photodynamic antimicrobial chemotherapy) has not yet found specific medical applications and still remains an open field of fundamental research.
Purpose: The assessment of the effect of a panel of seven tetraaryl-porphyrins, two commercial (PS **1** and **2**) and five synthetic (PS **3**–**7**) in in vitro experiments against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.
Methods: Three of the new photosensitizers (PS **3**, **4** and **5**) are tetracationic porphyrins and were prepared by *N*-alkylation of 5,10,15,20-tetra-4-pyridylporphyrin with a large excess of different benzyl chlorides; compound **7** is a dicationic porphyrin and was obtained in a similar way using a lower excess of 4-methoxybenzyl chloride. The neutral porphyrin (PS **6**) was previously described. Dose–response curves were obtained titrating the survivors of cell suspensions (10^8 cfu/ml) exposed to the PSs and irradiated with visible light (total fluence rate 266 J/cm^2).
Results: The non ionic porphyrin **6** was the least active PS against all the tested bacteria. Cationic PSs **3**, **4**, **5** and **7** were more active than the commercial **1** and **2**. The Gram positive *S. aureus* was more sensitive to all the PSs than the Gram negative *E. coli* and *P. aeruginosa*, the latter being the more resistant one. Compound **7** was found particularly efficient against *P. aeruginosa*, causing a 7 log units reduction of survivors at a concentration of 8 μM.
Conclusions: The reported results confirm that the presence of positively charged groups on porphyrin frame is fundamental for PSs antibacterial activity, however our data suggest that a moderate degree of lipophilicity, achievable by the introduction of aromatic hydrocarbon side chains on the pyridyl moieties, may improve PSs efficiency. Furthermore dicationic porphyrin **7** seems to be more efficient than the corresponding tetracationic derivatives thus emphasizing an interesting feature involved in the PSs activity.

Keywords: Porphyrin; Bacteria; PACT; Antimicrobial PDT; Photodynamic inactivation; *Escherichia coli*; *Pseudomonas aeruginosa*; *Staphylococcus aureus*

1. Introduction

Photodynamic antimicrobial chemotherapy (PACT), or antimicrobial photodynamic therapy (antimicrobial PDT), is a recently developed therapeutic option that utilizes photosensitive molecules and visible light to induce an oxidative damage to microbial pathogens [1]. This photodynamic approach could be a useful alternative to systemic medications in treating localized infections, thus avoiding the development of microbial resistance to systemic drugs. Easily accessible oral or skin infections could be good candidates for PACT treatment [2,3].

Among bacterial species which colonize human skin *Staphylococcus aureus* and *Pseudomonas aeruginosa* are

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doi:10.1016/j.jphotobiol.2006.04.003

infrequent resident flora, and they account for a wide variety of superficial as well as deep skin infections [4], such as pyodermas, impetigo, folliculitis, and ecthyma. Skin infection is the leading cause of death among patients hospitalised for burns; *S. aureus* (75%), *P. aeruginosa* (25%) and various coliform bacilli (5%) represent a relevant percentage of the pathogens isolated from burn wounds [5]. Peri-wound skin of pressure ulcers [6], diabetic foot ulcers [7] and other important dermatology diseases [3] can also be associated with infections.

Control of skin infections by chemotherapic agents is often jeopardized by the spreading of bacterial strains resistant to many conventional antibiotics; for example methicillin resistant *S. aureus* (MRSA) and multi-drug resistant *Pseudomonas* are increasing in burns units [5]. Furthermore, MRSA strains that exhibit a low-level resistance to a common biocide such as 2,4,4′-trichloro-2′-hydroxydiphenyl ether (triclosan) have also been isolated [8]. As photodynamic chemotherapy is a multi-target process, it is unlike to induce resistance in microorganisms [3].

In general, differently structured photosensitizers show a variable degree of bactericidal efficiency according to the organization of the cell wall: the presence of the outer membrane makes Gram negative bacteria more resistant to photoinactivation than Gram positive bacteria [9]. Gram negative bacteria can however be photoinactivated using PSs together with polycationic agents such as polymixin or Tris–EDTA; the effect of additives being the modification of the outer membrane permeability [10].

Among the several classes of known PSs, tetrapyrrolic derivatives have been widely studied in the last decade [1,11]. Synthetic *meso*-arylsubstituted porphyrins are particularly versatile starting materials to design new PSs as either ionic or non ionic moieties can be equally positioned on the periphery of the tetrapyrrole ring, thus modulating the photosensitizer polar character. Recent studies demonstrated that cationic porphyrins are more active than anionic or non ionic ones against both Gram positive and Gram negative bacteria, furthermore the cationic porphyrins are able to inactivate bacteria even in the absence of additives [12].

The aim of the present work is to investigate the in vitro photocytotoxicity associated with new synthetic *meso*-porphyrins (three tetracationic, one dicationic and one neutral) against *P. aeruginosa*, *S. aureus* and *E. coli*. The efficiency of the new porphyrins was compared with that of two tetracationic commercial compounds.

2. Materials and methods

2.1. General

UV–vis absorption spectra were measured on a Perkin–Elmer Lambda 10 instrument. ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer in $CDCl_3$ or [d_6] DMSO; chemical shifts are expressed in ppm relative to chloroform (7.26) and are reported as s (singlet), d (doublet), t (triplet), m (multiplet), bs (broad singlet). Mass spectrometric measurements were performed on a Finnigan LCQ-MS instrument. Elemental analyses were performed on a ThermoQuest NA 2100, C, H, N analyser, equipped with an electronic mass flow control and thermal conductivity detector. Analytical thin-layer chromatographies (TLC) were performed using Merck 60 F254 silica gel (precoated sheets, 0.2 mm thick) or on Macherey-Nagel F254 silica gel C_{18}-100 (precoated sheets, 0.25 mm thick). Silica gel 60 (70–230 mesh, Merck) was used for column chromatography.

2.2. Photosensitizers

The seven meso-substituted porphyrins used as PSs are reported in Fig. 1:

5,10,15,20-Tetra(*N*,*N*,*N*-trimethylanilinium)porphyrin tetratosylate (**1**).
5,10,15,20-Tetra(*N*-methyl-4-pyridyl)porphyrin tetratosylate (**2**).

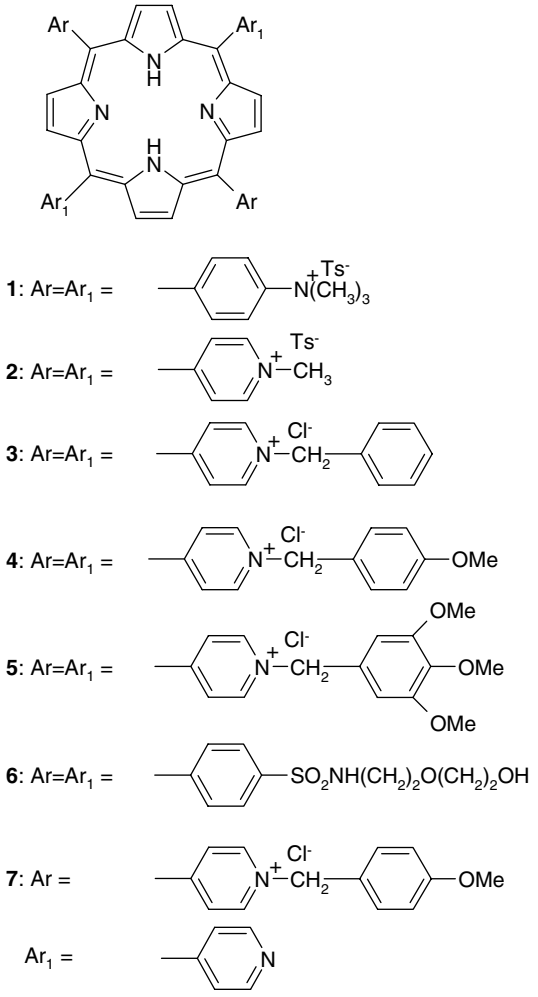

Fig. 1. Structure of the photosensitizers (**1**–**7**) used in the present work.