mycoses
Diagnosis, Therapy and Prophylaxis of Fungal Diseases

Review article

Photodynamic therapy for pathogenic fungi

Juliana Pereira Lyon,[1,2] Leonardo Marmo Moreira,[3] Pedro Claudio Guaranho de Moraes,[3] Fábio Vieira dos Santos[4] and Maria Aparecida de Resende[1]

[1]*Laboratório de Micologia, Departamento de Microbiologia, Instituto de Ciências Biológicas (ICB), Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil,* [2]*Departamento de Ciências Naturais (DCNAT), Universidade Federal de São João Del Rei (UFSJ), São João Del Rei, Minas Gerais, Brazil,* [3]*Departamento de Engenharia de Biossistemas (DEPEB), Universidade Federal de São João Del Rei (UFSJ), São João Del Rei, Minas Gerais, Brazil and* [4]*Universidade Federal de São João Del Rei (UFSJ), Divinópolis, Minas Gerais, Brazil*

Summary

Photodynamic therapy (PDT) is a minimally invasive approach, in which a photosensitiser compound is activated by exposure to visible light. The activation of the sensitiser drug results in several chemical reactions, such as the production of oxygen reactive species and other reactive molecules, whose presence in the biological site leads to the damage of target cells. Although PDT has been primarily developed to combat cancerous lesions, this therapy can be employed for the treatment of several conditions, including infectious diseases. A wide range of microorganisms, including Gram positive and Gram negative bacteria, viruses, protozoa and fungi have demonstrated susceptibility to antimicrobial photodynamic therapy. This treatment might consist of an alternative to the management of fungal infections. Antifungal photodynamic therapy has been successfully employed against *Candida albicans* and other *Candida* species and also against dermatophytes. The strain-dependent antifungal effect and the influence of the biological medium are important issues to be considered. Besides, the choice of photosensitiser to be employed in PDT should consider the characteristics of the fungi and the medium to be treated, as well as the depth of penetration of light into the skin. In the present review, the state-of-the-art of antifungal PDT is discussed and the photosensitiser characteristics are analysed.

Key words: PDT, Fungi, *Candida*, photosensitisers, antimicrobial photodynamic chemotherapy.

Photodynamic therapy

Photodynamic therapy (PDT) is a treatment that employs a photosensitiser compound, which is activated by exposure to visible light in a wavelength that is excitatory to this compound. The activation of the sensitiser drug results in several chemical reactions, such as the production of oxygen reactive species and other reactive molecules. The presence of these molecules in the site to be treated leads to the damage of target cells.[1]

Correspondence: Juliana Pereira Lyon, Rua Padre Machado, 152, Bela Vista, São João Del Rei, Minas Gerais, Brazil.
Tel.: +55 32 3371 4013. Fax: +55 32 3379 2408.
E-mail: julianalyon@yahoo.com.br

Accepted for publication 18 August 2010

Photodynamic therapy is a selective, non-invasive, or, at least minimally invasive modality of treatment for several types of diseases. In fact, PDT was first developed for the treatment of malignant diseases, and it has been successfully employed for the treatment of skin tumours,[1,2] cutaneous T-cell lymphoma[3] and for tumours localised in the oral cavity, blade and others.[4] Besides precancerous lesions, such as Bowen's disease, early stages of cervix cancer and Barrett's oesophagus can be treated with PDT.[5] However, in recent years, the range of indications for PDT has been expanding. This kind of treatment is also used for acne vulgaris and leishmaniasis, and for treating premature skin ageing due to sun exposure.[6] There are also lines of evidence that PDT can be applied against bacteria, fungi and viruses,[7] which will be discussed later.

Photodynamic therapy is performed in two stages. In the first step, the photosensitiser is administered to the

  doi:10.1111/j.1439-0507.2010.01966.x

patient as a cream, if the lesion is localised in the skin, or by injection into a vein, for inner lesions, although some drugs can be taken via oral, nasal or by pulmonary administration.[8] The drug must act for a time period to be concentrated in the target cells. Then, in the second stage, the light of the appropriate wavelength is applied though a light device, which is directly driven to the target in the case of skin lesions, or can be directed by an endoscope or a catheter to reach inner sites.

Regarding the light sources, lasers and non-coherent light sources are employed for PDT. An advantage of using lasers is that the light can be focused into fibre systems and led to otherwise inaccessible locations, such as urinary bladder, digestive tract or brain. For dermatology, however, non-laser sources are superior to laser systems because of their large illumination field, lower cost, smaller size, reliability and easy setup.[9]

Mechanism of action

The mechanism of action of PDT results from the interaction between visible light photons of appropriate wavelength with intracellular molecules of the photosensitiser. Reactive species are generated by the interaction between the light and the biological tissue causing an oxidative stress.

Oxidative stress has been defined as a disturbance in the pro-oxidant–antioxidant balance, in favour of the former, leading to potential damage. This imbalance may be due to an increased production of various reactive species and a decreased ability of the natural protective mechanisms of the organism to inhibit the action of these reactive compounds. Injury to cells occurs only when the reactive oxygen species overwhelm the biochemical defences of the cell.[1]

Photosensitiser compounds possess a stable electronic configuration, which consists of a singlet state in its ground energy level, i.e. there are no unimpaired electrons (diamagnetic electronic configuration). When the photosensitiser absorbs one photon of a specific wavelength, the electronic quantum jump occurs and the molecule is promoted to an excited state, which is also a singlet state, with short half life (10^{-6} to 10^{-9} s). The photosensitiser can return to the ground energy level through the emission of a photon, which consists of the fluorescence phenomenon or by internal conversion with loss of energy as heat by the interaction with neighbourhood molecules. Alternatively, the molecule can be converted to the triplet state (Fig. 1). This conversion occurs through an intersystem crossing, which involves a change in the electronic spin state.[10] The photosensitiser triplet state possesses a lower energy level than the singlet, consisting of a meta-stable state, and as consequence, showing a longer half-life.[10–12] The excited singlet state may interact with the surrounding molecules via type I reactions, whereas the triplet state interacts through type II reactions.[13]

Type I reactions lead to the formation of free radicals by hydrogen or electrons transference. These reactive species, after the interaction with oxygen, might produce oxygen reactive species, such as peroxide or superoxide anions, which attack cellular targets.[14] However, type I reactions do not necessarily require oxygen and could cause direct cellular damage by the action of free radicals. On the other hand, type II reactions need a mechanism to transfer energy from the triplet state of the sensitiser to the molecular oxygen, which usually occupies the triplet state $^3O_2$ in the characteristic electronic configuration of its ground state.[15] In any case, the life time of the reactive species is relatively low, implying that the representative damage

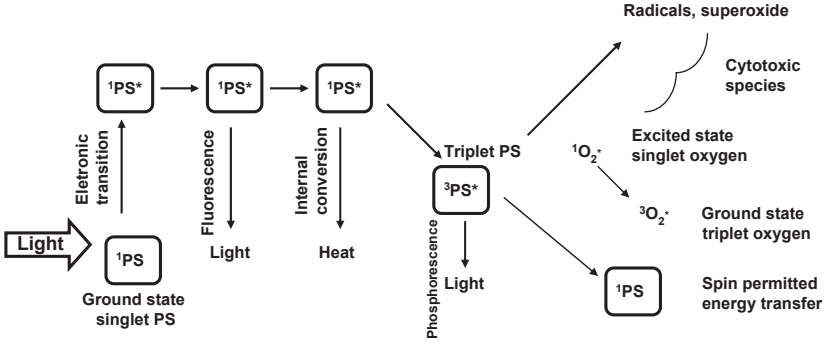

Figure 1 Schematic representation of the photochemical process of excitation of the photosensitiser (PS), including the possibilities of luminescence (fluorescence and phosphorescence), the singlet and triplet excited states and the reactive oxygen species generated by the energy transfer from the photosensitiser (PS).

action is focused on the target tissue, without affecting the neighbourhood tissues in a significant way.

Antimicrobial photodynamic therapy

The employment of PDT in the treatment of cancer and its effect in mammals' cells have been intensively studied.[16] Although the selective destruction of microorganisms by the action of light is known for more than a 100 years,[17] only recently, the susceptibility of microorganisms to this treatment has received a greater attention.[18] The technique can be employed against bacteria, viruses, fungi and parasites.[7,19–29]

Bacterial resistance to conventional antimicrobial chemotherapy is an issue of major concern, leading to the search for new therapeutic approaches. At this point of view, PDT arises as a promising strategy, as the mechanism of action involves multiple targets and mutations leading to resistance are unlikely to happen.

It is important to notice that PDT has been tested against skin and mucosal infections, where the drug delivery and the light application are easier to achieve. Two points must be considered when the efficacy of photodynamic procedure is evaluated: (i) the concentration of the photosensitiser in the target tissue; (ii) the intensity of photons incident on the target tissue.

Antifungal photodynamic therapy

The great interest in alternative therapies for the treatment of fungal infections comes from the fact that the number of antifungal agents available for chemotherapy is very restricted when compared with the number of antibacterial drugs. Furthermore, the cases of recurrent infections are a major issue for certain kinds of disease, such as candidiasis, dermatophytosis and chromoblastomycosis. Antifungal photodynamic therapy is a developing area of research,[10] and a majority of the literature in this area is concerned with *in vitro* experiments. Considering the potential of the technique in the treatment of fungal infections and the importance of developing new antifungal strategies, this is an area of great interest for future research studies.

PDT against *Candida* species

The photosensitisation of *Candida* yeasts inducing cellular damage through the utilisation of several sensitiser compounds has received special attention in several works.[19,30,31] *Candida* yeasts may cause skin and mucosal infection in patients with local predisposing conditions and are also a major cause of systemic infections, especially in immunocompromised patients.[32] The resistance of this yeast to azole antifungal agents has been increasingly reported.[33] The effect of PDT has been already demonstrated in the inhibition of germ tube formation,[30,34] biofilm formation[34,35] and reduction in adhesion to epithelial buccal cells.[36]

Although *Candida albicans* is the most prevalent species involved in human infections, other species are also important. It is worth mentioning that *Candida krusei* is intrinsically resistant to fluconazole.[37,38] Considering this fact, the action of PDT against different *Candida* species is very relevant. Dovigo *et al.* [35] evaluated the efficacy of PDT against *C. albicans* and *Candida glabrata* resistant to fluconazole, and against cells in suspension or in biofilms. These authors concluded that the fungicidal effect of PDT was strain-dependent and that although PDT was effective against *Candida* species, fluconazole-resistant strains showed a reduced sensitivity to PDT. In another study, Dovigo *et al.* [39] tested the efficacy of PDT with Photogen, a porphyrin photosensitiser, against four species of *Candida*. Interestingly, *C. krusei* was not inactivated by any of the associations between light and photosensitiser tested, while *C. albicans*, *Candida tropicalis* and *Candida dubliniensis* were completely eliminated.

Results achieved *in vitro* may not reflect the *in vivo* situation. It is known that biofilms are less susceptible to antimicrobial treatment than planktonic cultures. The same situation seems to occur in PDT. According to Dovigo *et al.* [35] biofilms were less susceptible to PDT than their planktonic counterparts. Another important point to be considered is the influence of the biological medium. Bliss *et al.* [18] observed that the uptake of Photophrin® (Axcan Pharma, Mont-Saint-Hilaire, QC, Canada) by *Candida* yeasts was poor when blastoconidia growth in nutrient broth, but was increased when cultures were in chemically defined medium. In addition, as expected, the uptake of photosensitiser influenced the susceptibility to PDT. Although the efficacy of PDT against *Candida* yeasts has been already demonstrated by *in vitro* studies, this point might impair its utilisation *in vivo*, and further studies are necessary.

It is worth mentioning that other photosensitisers may not have the same behaviour as that of Photophrin®. Indeed, Teichert *et al.* [40] achieved a drug-dependent photokilling of *C. albicans* in a murine model of oral candidosis employing methylene blue. On the other hand, Giroldo *et al.* [31] demonstrated that PDT with methylene blue increases membrane permeability in *C. albicans*, which could decrease the resistance of this

microorganism to other drugs. In this way, PDT could also be employed as a coadjutant to conventional antifungal chemotherapy.

PDT against other fungal species

Fungi can cause infections varying from superficial mycoses and cutaneous infections, to severe systemic diseases. Due to the easier application of light and drug delivery, the infections of the skin are more suitable to be treated with PDT.

The genus Malassezia can be responsible for a number of conditions. The most common infection is Pityriasis versicolor, but other diseases can also be mentioned, such as seborrheic dermatitis, folliculitis, neonatal pustulosis and blepharitis. Lee *et al.* [41] employed MAL-PDT (methyl 5-amino-levulinic acid) to treat patients with recalcitrant *Malassezia* foliculitis. Three from six patients achieved a strong improvement of the lesions after three sessions of PDT and one patient presented a moderate improvement.

A very interesting review was carried out by Calzavara-Pinton *et al.* [42] regarding the employment of PDT for the treatment of cutaneous fungal infections. According to this work, the preliminary results obtained *in vitro* are very promising and demonstrated that yeasts and dermatophytes can be sensitised by the administration of photosensitiser drugs, such as phenothiazines, phthalocyanines, porphyrins and the porphyrinic precursor aminolevulinic acid (ALA). In addition, the use of these sensitisers did not lead to the selection of resistant samples.

Dermatophytosis is very prevalent and the treatment frequently leads to recidives. These infections may cause great inconvenient to the patient and PDT could be a helpful alternative. In fact, dermatophytes have been evaluated in important research studies employing PDT. The effect of PDT has been observed against the dermatophyte *Trichophyton rubrum* by Smijs *et al.* [43]. These authors[44] also investigated the factors involved on the susceptibility of *Trichophyton rubrum* to PDT, employing two photosensitisers: 5,10,15-tris (4-methylpyridinium)-20-phenyl-[21H,23H]-porphine trichloride (Sylsens B) and deuteroporphyrin monomethylester (DP mme). It was observed that in acid medium with low levels of calcium (obtained by the addition of a chelant agent), the selective binding of Sylens B is enhanced. The same does not occur with DP mme. Calzavara-Pinton *et al.* [42] achieved promising results regarding the treatment of mycological lesions of the fingers in nine patients applying ALA as photosensitiser. This preliminary study might encourage future investigations on the use of PDT for the treatment of fungal infections of the skin.

Onychomycosis, the fugal infection of the nail, is one of the most difficult fungal infections to treat. Watanabe *et al.* [45] describe two cases of onychomycosis successfully treated with PDT with topical application of an ointment containing ALA 20%. These results are very important as not all cases of onychomycosis are healed by conventional antifungal chemotherapy.[45] Piracinini *et al.* [46] presented a patient with onychomycosis caused by *Trichophyton rubrum* to whom systemic antifungal agents were contraindicated, and the therapy with topical antifungal agents for 18 months had failed. Three sessions of PDT with ALA with intervals of 15 days led to the remission of the infection within a follow-up period of 24 months.

Some common environmental fungi may cause opportunistic infections in patients with predisposing conditions, which is the case of aspergillosis. Friedberg *et al.* [47] demonstrated an *in vitro* fungicidal effect for the photosensitiser Green 2W employed against *Aspergillus fumigatus*. These authors suggested that PDT could be an efficient option for the treatment of the cavitary lesions caused by this microorganism.

In this way, the treatment of fungal infections with PDT could be an interesting area of study, especially considering recurrent superficial and cutaneous mycological lesions. The treatment might be an alternative to conventional antifungal agents or a coadjutant to the traditional drug therapy.

Photosensitisers employed in PDT

Photosensitisers are necessary for PDT as well as a light source and the presence of significant concentrations of molecular oxygen in the target tissue. For this reason, more vascularised tissues generally achieve better results when submitted to PDT.[48] Some features are desirable on an ideal photosensitiser: absence of toxicity and toxic by-products; lack of mutagenic effect; selective accumulation on the target tissue, suitability for topical, oral and intravenous administration, and low cost.[48]

The major groups of photosensitisers employed in PDT are porphyrins, chlorines, phthalocyanines and phenothiazines. Mainly methylene blue and ortho-toluidine blue are the phenothiazines employed in PDT. Phenothiazines are cationic compounds, which have simple tricycle planar structures. The maximum absorption wavelength is 656 nm for methylene blue and 625 nm for ortho-toluidine blue.[10]

Porphyrins are tetraazamacrocycle compounds widely encountered in nature.[49,50] The optimal wavelength to photokilling is about 410 nm.[1] In turn, phthalocyanines and chlorines are porphyrin-like compounds that demonstrate longer wavelengths of absorption, near infrared (650–700 nm).[1] It is also relevant to mention that the ALA, which is not a photosensitiser itself, but a porphyrin precursor, is metabolised to protoporphyrin IX. ALA induced more pronounced protoporphyrin IX synthesis and accumulation in malignant and premalignant cells than in normal mammalian cells.[1]

Although there is a significant number of compounds that may act as photosensitisers, only a few are commercially available and approved for use in humans. Among these, we can cite the porphyrins Levulan® (Dusa Pharmaceuticals, Wilmington, DA, USA), Photophrin® and Vysudine® (QLT, Vancouver, BC, Canada), the porphyrin precussor ALA represented by Levulan® and Metvix® (Photocure ASA, Oslo, Norway), the chlorines Foscan® (BioLitec Pharma, Jena, Germany) Photochlor® (RPCI, Buffalo, NY, USA) and LS11® (Light Sciences, Snoqualmie, WA, USA), besides the Phthalocyanine Photosens® (General Physics Institute, Moscow, Russia).[1,48] These photosensitisers were especially developed for the treatment of malignant conditions. Currently, no clinical treatment based on antimicrobial PDT is licensed.[8]

When a photosensitiser is chosen for antifungal PDT, the light penetration is an important concern. Even if we consider infections of the skin, nails, hair, oral cavity, oesophagus or lower feminine reproductive tract, some degree of light penetration is required to kill fungi localised below the skin surface.[10] Light in the red region of the spectrum penetrates 3.0 mm down the tissue, whereas light in the blue region penetrates 1.5 mm. In this way, phthalocyanines and methylene blue are employed in a larger number of works, as they absorb near this desired wavelength.[10] Moreover, some fungi possess pigments that could interfere with light absorption, such as melanin, which is present in the dematiaceous fungi that cause chromoblastomycoses. In these cases, to obtain a photokilling effect, the photosensitiser employed must absorb light in a different wavelength from that corresponding to the absorption of the pigment present in the fungi. It is important to register that photosensitisers such as phthalocyanines and methylene blue have a maximum absorption wavelength above 600 nm. Considering this, the employment of these photosensitisers minimises the competition with the melanin maximum absorption wavelength. It is also known of the interference of compounds present in the biological medium, such as haemoglobin, and the choice of the photosensitiser is an important issue to consider for clinical application. These considerations allow observing that although the photodynamic antifungal therapy consists of a promising therapeutic alternative, there is a large field available for future research studies.

Conclusion

Photodynamic therapy is a minimally invasive approach, primarily developed for the treatment of malignant conditions. However, this therapy can be employed for the treatment of several diseases, including infectious diseases. The antifungal photodynamic therapy is a promising area of research and its development could benefit many patients, especially those with resistant or recurrent mycological infections of skin and mucosa. The antifungal action of PDT seems to be strain-dependent, and the influence of the biological medium must be taken into consideration because it can diminish the efficacy of the therapy *in vivo*. In addition, the photosensitisers to be employed in antifungal PDT must overcome fungal pigments and other substances that might be present in the medium to be treated, as well as the depth of penetration of light into the skin. Although positive results have been demonstrated *in vitro*, there are considerably fewer *in vivo* investigations. There are many fungal species to be evaluated. In addition, several issues must be improved in future research studies, such as the investigation of appropriate photosensitisers and drug delivery systems. Currently, no clinical treatment based on antimicrobial PDT is licensed. Considering this, antifungal PDT is an area of great interest for future studies, and advancements in this research should be strongly supported.

References

1 Moreira LM, Santos FV, Lyon JP, Maftoum-Costa M, Pacheco-Soares C, Silva NS. Photodynamic therapy: porphyrins and phthalocyanines as photosensitizers. *Aus J Chem* 2008; **61**: 741–54.

2 Bagnato VS, Kurachi C, Ferreira J, Marcassa LG, Sibata CH, Allison RR. PDT experience in Brazil: a regional profile. *Photodiagnosis Photodyn Ther* 2005; **2**: 107–18.

3 Calzavara-Pinton PG, Venturini M, Sala R. A comprehensive overview of photodynamic therapy in the treatment of superficial fungal infections of the skin. *J Photochem Photobiol B: Biol* 2005; **78**: 1–6.

4 Nakagawa H, Matsumiya T, Sakaki H *et al.* Expression of vascular endothelial growth factor by photodynamic

therapy with mono-l-aspartyl chlorin e6 (NPe6) in oral squamous cell carcinoma. *Oral Oncol* 2007; **43**: 544–50.
5 Trushina OI, Novikova EG, Sokolov VV, Filonenko EV, Chissov VI, Vorozhtsov GN. Photodynamic therapy of virus-associated precancer and early stages cancer of cervix uteri. *Photodiagnosis Photodyn Ther* 2008; **5**: 256–9.
6 Choudhary S, Nouri K. Elsaie photodynamic therapy in dermatology: a review. *Lasers Med Sci* 2009; **24**: 971–80.
7 Wainwright M. Photodynamic antimicrobial chemotherapy. *J Antimicrobiol* 1998; **42**: 13–28.
8 Cassidy CM, Tunney MM, McCArron PA, Donnelly RF. Drug delivery strategies for photodynamic antimicrobial chemotherapy: from benchtop to clinical practice. *J Photochem Photobiol B: Biol* 2009; **95**: 71–80.
9 Juzeniene A, Moan J. The history of PDT in Norway. Part II. Recent advances in general PDT and ALA-PDT. *Photodiagnosis Photodyn Ther* 2007; **4**: 80–87.
10 Donnelly RF, McCarron PA, Tunney MM. Antifungal photodynamic therapy. *Microbiol Res* 2008; **163**: 1–12.
11 Waris G, Ahsan H. Reactive oxygen species: role in the development of cancer and various chronic conditions. *J Carcinog* 2006; **5**: 1–8.
12 Weishaupt KR, Gomer CJ, Dougherty TJ. Identification of singlet oxygen as the cytotoxic agent in photoinactivation of a murine tumor. *Cancer Res* 1976; **36**: 2326–9.
13 Estevam ML, Nascimento OR, Baptista MS *et al.* Changes in the spin state and reactivity of cytochrome *c* induced by photochemically generated singlet oxygen and free radicals. *J Biol Chem* 2004; **279**: 39214–22.
14 Kalka K, Merk H, Mukhtar H. Photodynamic therapy in dermatology. *J Am Acad Dermatol* 2000; **42**: 389–413.
15 De Rosa FS, Bentley MVLB. Photodynamic therapy of skin cancers: sensitizers, clinical studies and future directives. *Pharm Res* 2000; **17**: 1447–55.
16 Dougherty TJ, Gomer CJ, Henderson BW *et al.* Photodynamic therapy. *J Natl Cancer Inst* 1998; **90**: 889–905.
17 Moan J, Peng Q. An outline of the hundred-year history of PDT. *Anticancer Res* 2003; **23**: 3591–600.
18 Bliss JM, Bigelow CE, Foster TH, Haidaris CG. Susceptibility of *Candida* species to photodynamic effects of photofrin. *Antimicrob Agents Chemother* 2004; **48**: 2000–6.
19 Zeina B, Greenman J, Purcell WM, Das B. Killing of cutaneous microbial species by photodynamic therapy. *Br J Dermatol* 2001; **144**: 274–8.
20 Zeina B, Greenman J, Corry D, Purcell WM. Cytotoxic effects of antimicrobial photodynamic therapy on keratinocytes *in vitro*. *Br J Dermatol* 2002; **146**: 568–73.
21 Zeina B, Greenman J, Corry D, Purcell WM. Antimicrobial photodynamic therapy: assessment of genotoxic effects on keratinocytes *in vitro*. *Br J Dermatol* 2003; **148**: 229–32.
22 Marotti J, Aranha AC, Eduardo Cde P, Ribeiro MS. Photodynamic therapy can be effective as a treatment for herpes simplex labialis. *Photomed Laser Surg* 2009; **27**: 357–63.
23 Rossi R, Bruscino N, Ricceri F, Grazzini M, Dindelli M, Lotti T. Photodynamic treatment for viral infections of the skin. *G Ital Dermatol Venereol* 2009; **144**: 79–83.
24 Kacerovska D, Pizinger K, Kumpova M, Cetkovska P. Genital warts treated by photodynamic therapy. *Skinmed* 2007; **6**: 295–7.
25 Akilov OE, Kosaka S, O'Riordan K, Hasan T. Parasiticidal effect of delta-aminolevulinic acid-based photodynamic therapy for cutaneous leishmaniasis is indirect and mediated through the killing of the host cells. *Exp Dermatol* 2007; **16**: 651–60.
26 Asilian A, Davami M. Comparison between the efficacy of photodynamic therapy and topical paromomycin in the treatment of Old World cutaneous leishmaniasis: a placebo-controled, randomized clinical trial. *Clin Exp Dermatol* 2006; **31**: 634–7.
27 Bristow C, Hudson R, Paget TA, Boyle RW. Potential of cationic porphyrins for photodynamic treatment of cutaneous Leishmaniasis. *Photodiagnosis Photodyn Ther* 2006; **3**: 162–7.
28 Akilov OE, Kosaka S, O'Riordan K, Hasan T. Photodynamic therapy for cutaneous leishmaniasis: the effectiveness of topical phenothiaziniums in parasite eradication and Th1 immune response stimulation. *Photochem Photobiol Sci* 2007; **6**: 1067–75.
29 Silva NS, Ribeiro Cde M, Machado AH, Pacheco-Soares C. Ultrastructural changes in *Tritrichomonas foetus* after treatments with AlPcS4 and photodynamic therapy. *Vet Parasitol* 2007; **146**: 175–81.
30 Munin E, Giroldo LM, Alves LP, Costa MS. Study of germ tube formation by *Candida albicans* after photodynamic antimicrobial chemotherapy (PACT). *J Photochem Photobiol B: Biol* 2007; **88**: 16–20.
31 Giroldo LM, Felipe MP, Oliveira MA, Munin E, Alves LP, Costa MS. Photodynamic antimicrobial chemotherapy (PACT) with methylene blue increases membrane permeability in *Candida albicans*. *Lasers Med Sci* 2009; **24**: 109–12.
32 Lyon JP, Costa SC, Totti VMG, Munhoz MFV, Resende MA. Predisposing conditions for *Candida* spp. carriage in the oral cavity of denture wearers and individuals with natural teeth. *Can J Microbiol* 2006; **52**: 462–7.
33 Lyon JP, Moreira LM, Cardoso MAG, Saade J, Resende MA. Antifungal suscetibility profile of *Candida* spp. oral isolates obtained from healthy individuals. *Braz J Microbiol* 2008; **39**: 668–72.
34 Chabrier-Roselό Y, Foster TH, Pérez-Nazario N, Mitra S, Haidaris CG. Sensitivity of *Candida albicans* germ tubes and biofilms to photofrin-mediated phototoxicity. *Antimicrob Agents Chemother* 2005; **49**: 4288–95.
35 Dovigo LN, Pavarina AC, Oliveira M *et al.* Fungicidal effect of photodynamic therapy against fluconazole-resistant *Candida albicans* and *Candida glabrata*. *Mycoses* 2009; doi: 10.1111/j.1439-0507.2009.01769.x. [Epub ahead of print].

36 Soares BM, Resende MA, Silva DL, Cisalpino PS. *In vitro* photodynamic inactivation of *Candida* spp. growth and adhesion to buccal epithelial cells. *J Photochem Photobiol B: Biol* 2008; **94**: 65–70.
37 Pinto PM, Oliveira RCBW, Lyon JP *et al. In vitro* antifungal susceptibility of clinical isolates of *Candida* spp. obtained from patients with different predisposing factors to candidosis. *Microbiol Res* 2008; **63**: 579–85.
38 Lyon JP, Resende MA. Evaluation of adhesion to buccal epithelial cells in *Candida* species obtained from denture wearers after exposure to fluconazole. *Mycoses* 2006; **50**: 21–24.
39 Dovigo LN, Pavarina AC, Ribeiro DG, Adriano CS, Bagnato VS. Photodynamic inactivation of four *Candida* species induced by Photogem. *Braz J Microbiol* 2010; **41**: 42–49.
40 Teichert MC, Jones JW, Usacheva MN, Biel MA. Treatment of oral candidiasis with methylene blue-mediated photodynamic therapy in an immunodeficient murine model. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002; **93**: 155–60.
41 Lee JW, Kim BJ, Kim MN. Photodynamic therapy: new treatment for recalcitrant Malassezia folliculitis. *Lasers Surg Med* 2010; **42**: 192–6.
42 Calzavara-Pinton PG, Venturini M, Capezzera R, Sala R, Zane C. Photodynamic therapy of interdigital mycoses of the feet with topical application of 5-aminolevulinic acid. *Photodermatol Photoimmunol Photomed* 2004; **20**: 144–7.
43 Smijs TGM, Schuitmaker HJ. Photodynamic inactivation of the dermatomycete *Trichophyton rubrum*. *Photochem Photobiol* 2003; **77**: 556–60.
44 Smijs TGM, Bouwstra JA, Schuitmaker HJ, Talebi M, Pavel S. A novel ex vivo skin model to study the susceptibility of the dermatomycete *Trichophyton rubrum* to photodynamic treatment in different growth phases. *J Antimicrob Chemother* 2007; **59**: 433–40.
45 Watanabe D, Kawamura C, Masuda Y, Yoichi A. Successful treatment of toenail onychomycosis with photodynamic therapy. *Arch Dermatol* 2008; **144**: 19–21.
46 Piraccini BM, Rech G, Tosti A. Photodynamic therapy of onychomycosis caused by *Trichophyton rubrum*. *J Am Acad Dermatol* 2008; **59**(5 Suppl.): S75–76.
47 Friedberg JS, Skema C, Baum ED *et al. In vitro* effects of photodynamic therapy on *Aspergillus fumigatus*. *J Antimicrob Chemother* 2001; **48**: 105–7.
48 Allison RR, Downie GH, Cuenca R, Hu X, Childs CJH, Sibata CH. Photosensitizers in clinical PDT. *Photodiagnosis Photodyn Ther* 2004; **1**: 27–42.
49 Moreira LM, Ribelatto JC, Imasato H. Conformações distorcida e planar do anel porfirínico em complexos e hemoproteínas: propriedades físico-químicas e implicações espectroscópicas. *Quím Nova* 2004; **27**: 958–63.
50 Magalhães JL, Moreira LM, Rodrigues Filho UP *et al.* Surface chemistry of iron tetraazamacrocycle on the aminopropyl-modified surface of oxidized n-Si(100) by AFM and XPS. *Surf Interface Anal* 2002; **33**: 293–8.