Photodynamic therapy as an antifungal treatment (Review)

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Abstract. Photodynamic therapy (PDT) involves the systemic or topical application of a photosensitizer (PS), alongside the selective illumination of the target lesion with light of an appropriate wavelength, in order to promote localized oxidative photodamage and subsequent cell death. Numerous studies have demonstrated that PDT is highly effective in the destruction of fungi *in vitro*. The mechanism underlying the effects of PDT results from the photons of visible light of an appropriate wavelength interacting with the intracellular molecules of the PS. Reactive species are produced as a result of the oxidative stress caused by the interaction between the visible light and the biological tissue. At present, no antifungal treatment based on PDT has been licensed. However, antifungal PDT is emerging as an area of interest for research.

Contents

1. Introduction

Photodynamic therapy (PDT) involves the systemic or topical administration of a photosensitizer (PS), alongside the selective illumination of a target lesion with light of an appropriate wavelength, in order to promote localized oxidative photodamage and subsequent cell death (1,2). PDT showed initial success in the treatment of malignant diseases, including skin tumors (3), cutaneous T-cell lymphoma (4) and cervical cancer (5,6), and precancerous lesions, including Bowen's disease (7,8) and Barrett's oesophagus (9). In recent years, PDT has also been used for the treatment of vulgaris, leishmaniasis, acne and bacterial, fungal and viral infections (10-12).

Previous studies demonstrated that PDT was highly effective in the destruction of fungi *in vitro* (13,14). At present, numerous antifungal drugs, including azoles, have a fungistatic (a delay in growth) rather than fungicidal (a complete inactivation of fungal conidia and hyphae) effect. Fungal conidia have been shown to be less susceptible to antifungal drugs, compared with hyphae (10,15).

It is estimated that 10-20% of the global population may be affected by mycoses, which are frequently recurrent and chronic (16). Acquisition of fungal pathogens results in significant morbidity causing discomfort, social isolation, disfigurement and may predispose one to bacterial diseases (17,18). However, fungi are eukaryotic organisms and their similarities to mammalian cells have led to significant difficulties in the development of new antifungal drugs. The heavy burden of fungal infections, and the increase in fungal strains resistant to the current antifungals globally (18), has rendered the development of new therapeutic strategies, such as antifungal photodynamic therapy, an urgent requirement.

2. PDT

PDT uses a PS and visible light of the appropriate wavelength to generate cytotoxic reactive species in the presence of oxygen. The presence of cytotoxic species in the target site results in the damage of target cells (19). PDT involves delivering visible light of the appropriate wavelength to excite the PS molecule to the excited singlet state (19).

The primary advantages of PDT are that the PS can be targeted to a specific cell or tissue and the visible light can be spatially directed to the infected area (19). In addition, the treatment of localized infections with PDT allows selectivity of the PS for microbes over host cells, delivery of the PS into the lesion and an ability to effectively illuminate the infected area (20).

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3. Mechanisms of PDT

The mechanism underlying the effects of PDT results from the photons of visible light of an appropriate wavelength interacting with intracellular molecules of the PS (21). Reactive species are produced as a result of the oxidative stress caused by the interaction between the visible light and the biological tissue, and cells are damaged when the reactive oxygen species overwhelm the biochemical defences of the cell (15). A PS is selectively delivered to the target microbial cells and activated by irradiation with light of the appropriate wavelength when taken up by these cells (22). When the PS is activated, type I and/or type II oxidative mechanisms may occur, which underlie the production of free radicals and singlet oxygen, respectively (23). The type I pathway involves electron-transfer reactions from the PS triplet state to a substrate, which results in the production of radical ions that may then react with oxygen to produce cytotoxic species, including superoxide as well as lipid-derived and hydroxyl radicals (24). The type II pathway involves energy transfer from the PS triplet state to ground state molecular oxygen (triplet) to produce excited-state singlet oxygen, which can oxidize various biological molecules, including nucleic acids, proteins and lipids (25,26). These reactive species may then inactivate microbes by damaging cellular components (25), predominantly via the photo-oxidation of nucleic acids, proteins (27) and membrane lipids (28).

The pathway that dominates (either type I or type II) is determined by the general circumstances, including the PS concentration, conditions in the cellular environment, the physicochemical characteristics of the PS and the chemical properties and morphology of the microbial target structures (29). The physiochemical properties of the PS determine its binding affinity to the cell wall of microorganisms; positively charged PS are typically more effective than negative or neutral ones, since, in the majority of cases, the outer surface of microorganisms is negatively charged (30). After the PS binds to the microbial wall, it may either remain outside the microorganism or be translocated to the inner cell membrane in order to induce light- and/or dark-stimulated wall permeability alterations (31). As well as exogenous-acting PS, protoporphyrin IX, which is produced from its precursor 5-aminolevulinic acid (ALA) in the heme biosynthesis signaling pathway, is an endogenous PS that is also important in antimicrobial PDT (32).

4. PS employed in PDT

A PS, a light source and the presence of significant concentrations of molecular oxygen in the target tissue are all required for PDT (33). The features of an ideal PS include the absence of toxicity, toxic by-products and mutagenic effects, an ability to selectively accumulate in the target tissue, a suitability for topical, oral and intravenous administration, and cost-effectiveness (34).

PS that are used in PDT include chlorines, porphyrins, phenothiazines and phthalocyanines. The phenothiazines used in PDT include orthotoluidine blue and methylene blue (35). Phenothiazines have simple tricyclic planar structures and are cationic compounds. The maximum absorption wavelength is 625 nm for orthotoluidine blue and 656 nm for methylene blue (35). Porphyrins are tetraazamacrocycle compounds that are widely encountered in nature (36). ALA is metabolized to protoporphyrin IX, thus it is not a PS, but rather a porphyrin precursor (2).

Light penetration is also important in PDT (36); light in the blue region penetrates 1.5 mm into the tissue, whereas light in the red region penetrates 3.0 mm. The optimal wavelength to promote photo-killing is ~410 nm (37).

The antifungal action of PDT appears to be strain dependent, and the type of biological medium has been shown to affect the efficacy of PDT *in vivo* (38). The PS to be used in antifungal PDT must be able to overcome fungal pigments and other substances, as well as the depth of penetration of light into the skin. However, no clinical treatment is currently licensed in the area of antimicrobial PDT (39).

5. PDT for fungi

The observed effects of PDT on yeasts and dermatophytes have led to the suggestion of its potential use for the treatment of skin mycoses (21). PDT is cost-effective, highly selective and avoids the occurrence of drug resistant strains (40). Therefore, PDT may become a valuable alternative to the already established antifungal drugs if the *in vitro* and *ex vivo* results can be transferred to clinical practice (41).

Trichophyton rubrum causes persisting dermatophytosis, and patients with a compromised immune system may suffer from chronic dermatophytosis (42). The ability of the host's defence mechanisms to overcome a dermatophytic infection has been closely associated with the appearance of the infection. The fungal wall is associated with virulence and is also the frequent target of numerous antifungal agents (43). The outermost layer of the fungal wall consists of β-glucan, the second layer contains galactomannans and complex glycoproteins attached to a peptide backbone, and the third layer consists primarily of chitin, which gives the fungal wall its rigidity (44,45). The innermost layer of the wall is the cell membrane (46). Typically, it takes conidia ~2 h to adhere to the skin surface (47). Following the initial attachment to keratinized structures, the conidia germinate and form hyphae, which penetrate the epidermal layer (48). The optimum pH of the proteinases and some of the keratinases produced by the fungi during this initial stage is acidic (49), corresponding to the pH of the skin surface in humans. However, *in vitro* studies with *T. rubrum* have shown that the pH of the cultivation medium changes as a function of nutrients used to reach values of pH 8-9 (50). Proteolytic and keratinolytic activity appear to be important virulent factors for dermatophytes.

Typically, the treatment of dermatophytoses involves the administration of an antifungal drug. However, oral antifungal agents may induce side-effects, including hepatotoxicity, and may interact with other drugs (50).

It is important to note that PDT has previously been investigated for the treatment of skin and mucosal infections (50,51). The concentration of the PS in the target tissue and the intensity of photons directed at the target tissue must be considered when evaluating the efficacy of the photodynamic procedure (51). *Candida* yeasts may cause skin and mucosal infections in patients with local predisposing conditions and are also a major cause of systemic infections, particularly in

patients with a compromised immune system (52). Studies have demonstrated that PDT was able to inhibit germ tube and biofilm formation, and reduce adhesion to epithelial buccal cells (53). Dovigo *et al* (54) reported that biofilms were less susceptible to PDT, as compared with their planktonic counterparts. The effect of PDT has been observed against the dermatophyte *T. rubrum* (25), and two cases of onychomycosis successfully treated with PDT have previously been described, which involved topical application of an ointment containing 20% ALA.

6. *In vitro* studies

The majority of published work on antifungal PDT has centred on *in vitro* laboratory investigations, involving the use of various fungi, PS and irradiation protocols (55,56). At present, there have been no reports on the development of resistance to antifungal PDT, and the treatment has not been associated with mutagenic effects or genotoxicity. The effects of PDT have predominantly been observed against the dermatophyte *T. rubrum* (57,58).

7. *In vivo* studies

The clinical efficacy of ALA-PDT in the treatment of fungal infections of human skin has previously been investigated (57). Mutagenic effects of photodynamic treatment with chloroaluminum phthalocyanine and RPL068 were not found in *Kluyveromyces marxianus* (58) nor *Candida albicans* (59). Following a primary search of 106 articles on databases including, MEDLINE, EMBASE and the Cochrane Library to evaluate the efficacy and safety of PDT for superficial mycoses, it was determined that only seven papers involving 63 patients with superficial mycoses were included. The PS used in all patients was 20% ALA (42).

8. Adverse effects of ALA-PDT

The overall tolerability of ALA-PDT has been shown to be good, although the adverse effects of ALA-PDT for treating superficial mycoses included a burning sensation during irradiation, erythema, pain, edema and blistering (57).

9. Limitations and improvement of ALA-PDT

ALA-PDT can be used as a long-term treatment without causing the accumulation of protoporphyrin IX in normal skin (60). ALA is a hydrophilic, zwitterionic molecule with a molecular weight of 167.6 g/mol (61). It is difficult for ALA to penetrate through intact skin (62,63); therefore, improving delivery systems for ALA in the skin will have an important role in the clinical application of ALA-PDT. The enhancement of ALA skin penetration may include physical methods, such as ultrasound, laser, microneedles and iontophoresis, the addition of chemical penetration enhancers, including oleic acid and dimethyl sulfoxide, or the use of lipophilic ALA derivatives or various vehicles to improve the transdermal delivery of ALA (64). Lipophilic ALA ester derivatives may have an enhanced potential for clinical use (65,66). In previous studies, several strategies were used in order to improve ALA penetration into the skin, including iontophoresis (67), lasers (68), microneedles and ultrasound (69).

Mechanisms of penetration enhancers include disruption of the highly ordered structure of stratum corneum lipids, interaction with intercellular proteins and improved partitioning of the drug, co-enhancer, or solvent into the stratum corneum (69). Electron microscopy revealed that a discreet lipid domain is induced within the stratum corneum lipid bilayers upon exposure to oleic acid, which enhanced the permeation of drugs across the skin (54) Friedberg *et al* (70) reported that oleic acid was able to optimize the skin delivery of ALA in PDT.

The half-life of ALA in the body is ~45 min (70). Vehicles may serve as a solubilization matrix (71). Liposomes, which are microscopic vesicles consisting of one or more membrane-like phospholipid bilayers surrounding an aqueous medium (72,73), are one of the best drug delivery systems for low molecular-weight drugs, such as ALA (74,75).

At present, PDT is used for the prevention and treatment of a variety of malignant skin tumors and inflammatory diseases, including non-melanoma skin cancer, actinic keratoses, acne vulgaris, photorejuvenation, and hidradenitis suppurativa (76,77).

In vitro studies demonstrated that ALA was sufficiently metabolized into protoporphyrin IX and was able to effectively kill *T. rubrum* and *C. albicans* (77).

10. Conclusions

PDT includes the systemic or topical administration of a PS, alongside the selective illumination of a target lesion with light of the appropriate wavelength, in order to cause localized oxidative photodamage and subsequent cell death. Numerous studies have demonstrated that PDT is highly effective in the destruction of fungi *in vitro*. However, at present, no clinical treatment based on PDT has been licensed. The current study presents *in vitro* and *in vivo* and human studies that support antifungal PDT as a new approach against mycoses. In conclusion, antifungal PDT is emerging as an area of interest in the discovery of novel antifungal therapeutic strategies.

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