Photodynamic Therapy for the Management of Onychomycosis: A Promising Strategy

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Editorial

Onychomycosis is a fungal infection of the toenails and fingernails that results in thickening, discoloration, splitting as well as lifting of the nails from the nail bed. It affects 14% of the total world population, with more prevalence in elders and diabetics. Both dermatophytes (*Trichophyton rubrum* or *Trichophyton mentagrophytes*) and nondermatophytes (*Scopulariopsis brevicaulis, Aspergillus spp, Fusarium spp,* and sometimes *Candida spp)* have been identified as etiologic agents of onychomycosis. The treatment of onychomycosis is known to be challenging since it is chronic, difficult to eradicate and tends to relapse. Multiple therapies, including surgical, chemical, topical, and oral methods, have been described for its treatment. However, several factors like the difficulty of achieving penetration of the nail plate, lack of adherence to treatment (which lasts for months), the poor response of some fungi to antifungals, and individual susceptibility lead to poor treatment outcomes [1]. The complete cure comprised of clinical cure (implying nail clearing) and mycological cure (both negative microscopy and dermatophyte culture) is often unattainable [2]. Therefore, there is a need to expand treatment options and reduce the adverse effects associated with the conventional therapies. The photodynamic therapy (PDT) is a one of the interesting option that can help to overcome the limitations described above.

PDT is a clinical treatment that combines the effect of visible light irradiation with subsequent biochemical events that arise from the presence of a photosensitizing drug to cause destruction of the selected cells. The photosensitizer, when introduced into the body, accumulates in the target cells and a measured light dose of appropriate wavelength is then used to irradiate the target tissue [3]. This activates the drug through a series of electronic excitations and results in a series of cytotoxic reactions, which can either be dependent or independent of the generation of reactive oxygen species [4]. PDT has progressed considerably from early application of sunlight and hematoporphyrin derivative to the use of Photofrin˚ and to second generation preformed photosensitizers and topical application of prodrugs like 5-aminilevulinic acid (ALA) and methyl-aminolevulinic acid (MAL) which leads to *in situ* synthesis of protoporphyrin IX (PpIX).

Topical PDT is a well-established treatment in dermatology and it has proved to be a useful therapy for a variety of malignant skin tumors, psoriasis and inflammatory diseases [5]. Moreover, PDT has been investigated for several infective viral and bacterial skin diseases both *in vitro* and *in vivo* [6,7]. In cases involving superficial fungal infections of the skin, several *in vitro* studies have demonstrated the effectiveness of PDT against cultured *T. rubrum* in suspension culture [8,9]. Recently, Smijs *et al.* [10] reported the effectiveness of PDT for *T. rubrum* infection in an *ex vivo* human skin model. In light of these findings, researchers are now showing interest in ALA PDT for the treatment of onychomycosis [10].

The attractiveness of PDT as a therapeutic option for the management of onychomycosis is very obvious due to its number of inherent advantages like a broad spectrum of action, effectiveness independent of patterns of antimicrobial resistance, photo-inactivation of the microorganisms, availability of formulations that allow specific delivery of the photosensitizer to the infected area and spare adjacent healthy tissue, use of low cost light sources to activate a photosensitizing agent and compatibility with other antibiotic and antifungal drugs in combination therapies.

In vitro Studies

As antifungal, PDT is a rapidly developing technique; the vast majority of published work has focused on *in vitro* laboratory investigations. Various fungi, photosensitizers and irradiation protocols have been employed. In most cases, complete inhibition of both yeasts and dermatomycetes has been readily achieved. Critically, no reports on development of resistance to antifungal PDT

Table 1: Various *in vitro* studies employing PDT carried out by different researchers.

Microorganism	Photosensitizer	Light Administration				Remarks	Ref.
		Source	Wave-length (nm)	Dose (J/cm²)	Fluence Rate (mW/cm²)		
T. mentagrophytes M. gypseum	Methylene blue, neutral red, proflavine hemisulfate	Blue light	455	~ 1.1	30	Complete fungicidal effect with proflavine	[11]
T. rubrum T. verrucosum T. violaceum M. canis M. gypseum Epidermophyton floccosum (spore solution)	Hematoporphyrin derivative, methylene blue, toluidine blue	Oriel sun simulator	Not mentioned	72-144	40	Fungicidal effect with hematoporphyrin and methylene blue at 10⁻³ M for *M. canis, T. mentagrophytes* and *T. verrucossum*	[12]
T. rubrum (liquid culture medium)	Sylsens B DP mme	Massive (no. 74900/21). 13 max. 500 W-230V-R7s, IP44	Not mentioned	108	30	Fungicidal (3 g / mL) effect with Sylsens B and DPmme, while, fungistatic effect was observed for 1 week with phthalocyanines and Photofrin	[8]
T. rubrum (suspension culture of hyphae and microconidia)	Sylsens B DP mme	Massive (no. 74900/21). 13 max. 500 W-230V-R7s, IP44 with a cut-off filter at 600 nm	580-870	108	30	Fungicidal effect for microconidia was found with Sylsens B (1 μM) and DPmme (>5 μM), while fungicidal for hyphae was observed with Sylsens B (10 μM) and DPmme (40 μM)	[13]
T. rubrum (liquid culture medium)	ALA (1-10 mmol l−1)	Quartz-halogen lamp Zeiss KL 2500 LCD	Not mentioned (white light)	128	36.8	Fungistatic action with reductions in the number or the diameter of the colonies	[9]
T. Interdigitale, C. albicans	ALA (0-100 mM)	Paterson Lamp (Phototherapeutics Ltd.)	635	100	100	ALA showed fungistatic effect with 79% reduction in viability	[14]
T. rubrum	Toluidine blue O	Light Emitting Diode (LED)	630	18-90	Not specified	Fungicidal effect at a dose of 25 μM when irradiated with the energy density of 72 J/cm²	[15]
T. rubrum	Toluidine blue	LED	630 nm	48	Not specified	Fungicidal effect was observed with 98% of fungal inhibition	[16]
T. rubrum T. mentagrophytes	Hypericin	LED	602	37	10.3	3-log fungicidal effect with hypericin concentration ranges of 10-50 μM	[17]
T. rubrum	Rose Bengal	Three 3-watt H-HP803 PG LED modules	530	24	13.4	100% inhibition was achieved at a dose of 140 μM	[18]

currently exist and the treatment is not associated with mutagenic effects or genotoxicity in either fungi or cultured human cells [11-18]. The antifungal effectiveness of PDT has been assessed, *in vitro*, in different types of fungi using several different photosensitizers at different concentrations and light sources at various wavelengths (450-870 nm). Phenothiaziniums, the porphyrins and phthalocyanines are the most extensively investigated classes of photosensitizers used for *in vitro* antifungal studies. Table 1 summarizes such *in vitro* studies carried out by different researchers.

In vivo Studies

The promising *in vitro* findings of PDT have raised interest of researches in *in vivo* studies. However, till now there is little clinical experience with the use of PDT in the treatment of onychomycosis and no standardized protocol exists. Table 2 enlists the cases reported and clinical trials published till now. Most of these studies include patients in whom previous antifungal treatments had failed as well as patients who had underlying diseases with denial of oral treatment.

In all the studies, a light source that emitted a wavelength in the

red spectrum, which is not absorbed by hemoglobin but can penetrate more deeply into living tissue, was used. The lamp most often used in these studies was light emitting diode (LED) with a wavelength of 630 ± 10 nm (Aktilite). LEDs are compact, require less energy to emit light at the desired wavelengths, do not cause thermal damage to biological tissues, and are made to produce multiple wavelengths. Although its effect *in vitro* is fungi static, the photosensitizing agent most commonly used in the literature was 20% ALA [19,20] or its derivative i.e. 16% methyl-aminolevulinate (MAL) [21-24]. Both have been shown to be effective when applied topically, and have completely disappeared from the treated tissue within 24 to 48 h of application. Other photosensitizers used were 2% methylene and a hematoporphyrin derivative (Photogem) [25,26].

Most authors report a clinical and microbiological cure rate of 90% to 100% following treatment; however, this percentage decreases on follow-up. The reason behind the decrease in cure rate during follow up is still unknown. The efficacy of PDT was also observed to be dependent on the pretreatment of the nail with urea and/or mechanical abrasion to increase its permeability to the

Table 2: *In-vivo* studies of Onychomycosis Treated with Photodynamic Therapy.

Type of onychomycosis	Site of infection	Causative Organism	Pre-treatment	Photo-sensitizer	Light Administration (Source/Wavelength (nm)/Dose (J/cm^2/ Fluence rate (mW/cm^2)	Incubation Time	No. of sessions	Follow up period (months)	Rate clinical or Mycological Cure	Ref.
Subungual, distal and lateral	Nail of first toe	Not specified	20% urea	20% ALA	Pulsed excimer dye laser; 630; 100; Not specified	5 h	6-7	3 and 6	100 % clinical and mycological cure was observed and no recurrence was found at follow-up visit.	[19]
Total onychomycosis and proximal subungual	Right big toenail and left big toenail	*T. rubrum*	40% urea	5-ALA (160 mg/g)	Akilite; 630; 37; Not specified	3h	3	Every 3 months for 24 months	Neither mycological nor clinical cure was achieved until last follow up	[20]
Toenail onychomycosis	Not specified	Not specified	None	MAL	LED; 633;37; Not specified	15 min	1	4	Negative culture and appreciable nail improvement was observed after the follow up period	[21]
Subungual, distal and lateral	Nail of first toe (22 patients) Other toenail (8 patients)	*T. rubrum*	20% urea + mechanical abrasion	20% ALA	Waldmann PDT 1200; 570-670; 40; 40	3 h	3	12 and 18	At 12 months follow-up 43% were cured, however, at 18 months follow-up the cure rate dropped to 36.6%	[22]
White superficial	5th fingernail	*Acremonium sclerotigenum*	None	16% MAL	Aktilite; 630; 37; Not specified	4h	3	3, 6, 9, and 12	The patient achieved mycological and clinical cure and remained asymptomatic after 12 months of follow up.	[23].
Onychodystrophy, White superficial	4th fingernail, 1st to 5th fingernails	*F. oxysporum, Aspergillus terreus*	40% urea	16% MAL	Aktilite; 630; 37; Not specified	4h	3	6	100 % cure rate was observed	[24]
Subungual, distal and lateral. Onychodystrophy	First toenail both feet both feet	Not specified	20% urea + Mechanical abrasion	Hematoporphyrin derivative (Photogem 1 mL, mg/mL)	LED; 630; 54; Not specified	1h	6	None	100 % cure was observed after the therapy	[25]
Subungual, distal and lateral	Not specified	*T. rubrum, T. mentagrophytes E. floccosum A. niger Candida* sp. *Fusarium* sp.	Mechanical abrasion	2% methylene blue aqueous solution	LED; 630; 18; 100	3 min	12	1 and 12	At the end of treatment 90% cure was obtained, which dropped to 80% at 12 months follow-up	[26]
Subungual, distal and lateral	Not specified	T. rubrum	Mechanical abrasion	2% methylene blue aqueous solution	LED; 630; 36; 100	3 min	12	1 and 12	The clinical response was found to significantly better in patients with mild-to-moderate (100%) onychomycosis compared with patients with severe onychomycosis (63.6%)	[27]

photosensitizing agent [19] and active removal of hyperkeratosis [22].

Drug Delivery Studies

The majority of published PDT studies have aimed at *in vitro* investigations elucidating type of photosensitizer, doses and wavelength of light effective in the photodynamic inhibition of yeasts and dermatophytes. However, in order to move PDT from the laboratory to the clinical studies, drug delivery systems have been formulated by few researchers. In addition, investigations must be done to determine the clinical performance of such delivery systems; howbeit, a very small number of studies of this kind have been published till date.

Donnelly et al. [14] studied the *in vitro* penetration of ALA from a bioadhesive patch containing ALA (50mg cm^{-2}) across human nail and into neonate porcine hoof. PDT is thought to be useful for the treatment for onychomycosis if sufficient concentrations of drug could be achieved within the nail matrix and at the nail bed. Patch application for 24 h and 48 h allowed an ALA concentration of 2.8 mm and 6.9 mm, respectively to be achieved on the ventral side of excised human nail. However, application time of 24, 48 and 72 h showed no significant effect on the ALA concentration at mean depths of 2.375 mm in neonate porcine hoof as concentration of 0.1 mm was achieved irrespective of time period.

Smijs *et al.* [10] used an *ex vivo* human skin model to investigate the ability of porphyrins to kill *T. rubrum*. The photosensitisers, in liquid vehicles, were applied to the skin, previously inoculated with the dermatomycete. Observations revealed that short incubation times (8 h) gave complete inhibition upon irradiation (108 J cm^{-2}, 580–870 nm), while incubation for longer times (> 24 h) prior to irradiation yielded no inhibition. Further, water was found to be more effective delivery vehicle, in terms of killing rate as compared to the cell culture medium Dulbecco's Modified Eagles's Medium (DMEM). This may be attributed to the lower the pH of the water (5.2) used in the study than that of DMEM (pH 7.4). A lower pH might have promoted a selective binding of cationic photosensitizer, i.e. Sylsens B to the fungus rather than to the SC resulting in higher inhibition rate [10].

Conclusion

PDT is a useful therapy in dermatology e.g. in malignant skin tumors, psoriasis and inflammatory diseases etc. No PDT system is FDA-approved for treating onychomycosis till date, however, recent research has elucidated promising role of PDT in the treatment of onychomycosis with resultant benefits for patients as well as clinicians. Moreover, the topical nature of PDT obliterates systemic side effects and drug- drug interactions associated with conventional oral therapy.

Following a number of successful clinical trials and pilot studies, now there is a requirement to carry out extensive multisite clinical studies of PDT so as to improve the therapeutic outcomes for the patients and also to upgrade its FDA status.

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