Small Scale Trial of Photodynamic Treatment of Onychomycosis in São Paulo

Joao Paulo Tardivo[1], Mark Wainwright[2*], Mauricio Baptista[3]

[1]Fundação Medicina ABC, Santo André, SP, Brazil.

[2]School of Pharmacy & Biomolecular Sciences, Liverpool John Moores University, Liverpool, United Kingdom L3 3AF.

[3]Departmento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, SP, Brazil.

*Author for correspondence, email mark_wainwright@hotmail.com

Abstract

The use of methylene blue and toluidine blue in the photodynamic treatment of onychomycosis at an ambulatory clinic in São Paulo, Brazil is reported. Local application and illumination of infected nails produced a response in 53 of 62 patients, without any pain or burning associated with the therapy.

Keywords: methylene blue; onychomycosis; photoantimicrobial; photodynamic; photofungicidal; toluidine blue.

1. Introduction

Among infectious diseases, external fungal infections in the non-immunocompromised host are generally treated as insignificant. While visible lesions, e.g. of the hands or face, might require treatment on the basis of cosmesis, those of the feet – at least in temperate climes – are likely to be mainly hidden by clothing and are consequently often ignored. Non-intervention against dermatophytic fungi, such as the *Trichophyton* spp involved in Athlete's Foot, allows fungal spread and consequent invasion of the nail bed (onychomycosis). Whereas infection of the dermis is normally susceptible to conventional antifungal therapy, either topically or systemically administered, nail bed infection represents a difficult presentation for such topical approach and often requires recourse to extended systemic administration of azole or allylamine drugs [1].

From previous *in vitro* work, it is clear that cationic photosensitising agents, such as the established methylene blue derivatives, offer a rapid fungicidal effect, due to the production of reactive oxygen species (e.g. singlet oxygen) on illumination [2]. In addition, methylene blue itself and other phenothiazinium derivatives, e.g. toluidine blue, have established low toxicities in the human host [3]. This combination therefore suggests a potential therapeutic approach to the topical treatment of recalcitrant fungal infection, such as that presented in onychomycosis.

The following report describes a recent and ongoing small-scale trial of the use of methylene blue and toluidine blue in combination as an alternative to the conventional treatment of fungal nail bed infection in patients in a São Paulo clinic. It should be noted that this was not intended as a properly controlled clinical trial involving controls and placebos. The treatment was offered as an alternative to conventional chemotherapeutics for patients presenting with onychomycosis.

2. Materials and Methods

2.1 Photosensitiser preparation

Solutions of methylene blue (MB) and toluidine blue (TB) (Labsynth Laboratory Suppliers, São Paulo, Brazil) were prepared at 2% (w/v) in 10% aqueous ethanol (v/v).

2.2 Light irradiation

The in-house light source RL50® [4] was employed. This emits light in the red region of the spectrum from 600-750 nm and overlaps well with the MB/TB action spectra. It delivers 100mW/cm^2 at a distance of 5 cm from the source.

2.3 Patients

Patients with onychomycosis were treated in the Dermatology Service of the School of Medicine of ABC, São Paulo, as a free public service. Informed consent was obtained from the patients.

2.4 Protocol

Infected nails were photographed before treatment. The nail bed was cleaned after scraping the nail surface with a sharp curette and the material removed was sent for microbiological analysis. A 1:1 volume of the 2% methylene blue / 2% toluidine blue mixture was then applied between the nail plate and nail bed. After 5 minutes the nail was irradiated superficially with the RL50 source at a distance of 5 cm for 3 minutes, providing a final irradiance of 18 J/cm^2. The site was assessed and photographed after a period of 30 days, when the protocol was repeated if necessary.

3. Results and Discussion

Microbiological analysis of the initial nail curettage showed that the infections were caused (singly) by the common fungi *Trichophyton rubrum*, *T. mentagrophytis* or *Candida albicans*. The authors have reported the successful photodynamic eradication of these organisms previously *in vitro*, using MB, TB and related phenothiazinium derivatives as the photosensitising agents [5,6].

From Table 1 it can be seen that 28/62 patients completing the treatment exhibited complete clearance of fungal nail infection (Figure 1), with 25/62 partial clearances.

Outcome	**n**	**%**
Complete clearance	28	45
Partial clearance	25	40
No change	9	15

Table 1. Outcomes from the treatment of onychomycosis patients (n = 62).

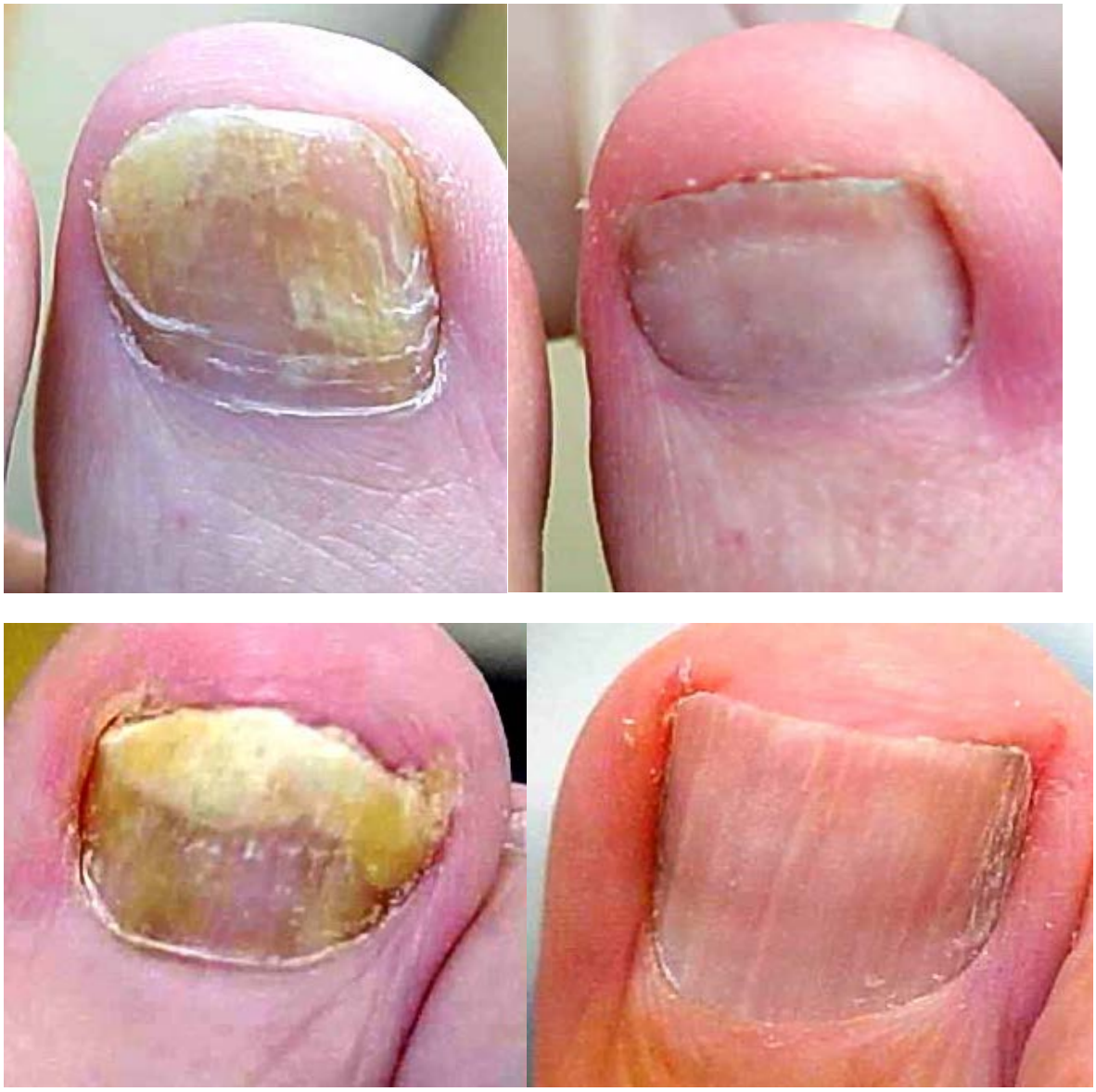

Figure 1. Complete clearance of nailbed infection after photodynamic treatment.

The data in Table 1 represent the initial results from an ongoing treatment protocol carried out in São Paulo since 2000, with the number of sessions varying with the severity of disease and speed of nail growth. As noted, 62 patients were considered to have completed therapy. Treatments often covered multiple visits and several patients dropped out due to dislike of the blue coloration of the nail and peripheral tissues (Figure 2). The number of sessions ranged from 1 to 22, and sessions occurred once a month. The average number of treatments for complete clearance was five (5.22) and some of the patients showing partial clearance are still under treatment. However, of those treated, none reported any pain, burning or discomfort during the illumination period, in contrast to other local photodynamic anti-infective approaches: for example, the treatment of acne vulgaris using aminolaevulinic acid [7]. Patients in the present cohort had generally tried to cure their infections previously using conventional antifungal agents, such as ketoconazole or terbinafine.

However, there was no direct evidence to suggest that the causative organisms were of conventional-drug resistant strains. Theoretically at least, and in agreement with *in vitro* reports, the conventional drug-susceptibility of the invading organism should have no influence on the effectiveness of the photosensitiser, due to the absence of applicable resistance mechanisms to ROS [8].

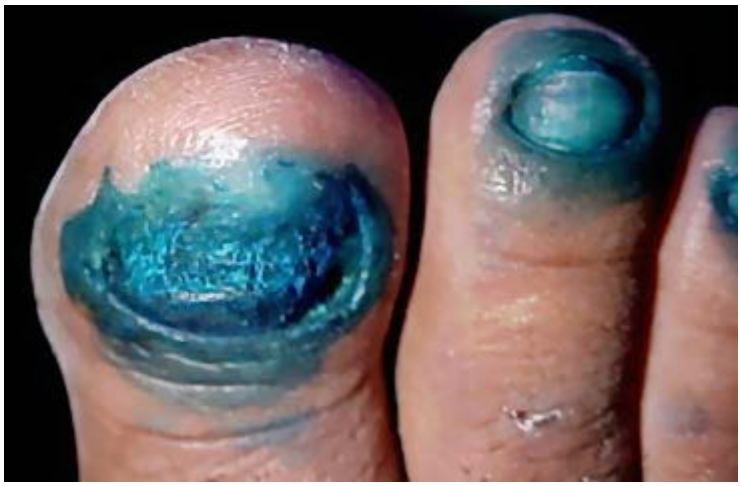

Figure 2. Application of methylene blue/toluidine blue, showing staining of peripheral tissue.

The photodynamic approach to the treatment of fungal disease contrasts strongly with those employing conventional agents. Firstly, at present, medical supervision is required to oversee the application of both the photosensitiser and light to the infected area. However, the photodynamic approach might require only single application. This is clearly not the case with conventional therapeutics: although oral antifungal therapy must be prescribed and thus requires the involvement of medical personnel, administrative regimens are often extensive and prolonged, requiring up to sixteen weeks' oral administration. In addition, significant side effects have been reported during terbinafine self-administration, including liver damage, taste and smell disturbances and depressive symptoms [9].

The authors have reported the use of the methylene blue approach also for the treatment of herpes simplex lesions, which again exhibited ease of use, absence of pain/burning and excellent cosmesis [10]. Since the general method has no apparent negative sequelae, repeat applications might be made by the patient, although this would presumably require a metred light source. It is expected that relatively simple localised infections of this type would be amenable to the photodynamic approach in future, in order to conserve valuable conventional antimicrobial agents.

4. Conclusion

Given the widespread therapeutic problems and increased healthcare expenditure due to microbial drug resistance, it is sensible to examine novel, non-conventional approaches to infection control. Clinical photodynamic antimicrobial chemotherapy, such as that described here, offers effective antifungal capability against a target which normally requires considerable effort on the part of the patient, due to the extended administration period required, with concomitant rates of treatment failure.

References

1. R. Hay, Superficial fungal infections, Medicine 41 (2013) 716-718.

2. I.O.L. Bacellar, C. Pavani, E.M. Sales, R. Itri, M. Wainwright, M.S. Baptista, Membrane damage efficiency of phenothiazinium photosensitizers, Photochem. Photobiol. 90 (2014) 801-813.

3. N. Kashef, G.R.S. Abadi, G.E. Djavid, Photodynamic inactivation of primary human fibroblasts by methylene blue and toluidine blue, Photodiag. Photodyn. Ther. 9 (2012) 355-358.

4. J.P. Tardivo, A. Del Giglio, L.H. Paschoal, A.S. Ito, M.S. Baptista, Treatment of melanoma lesions using methylene blue and RL50 light source, Photodiag. Photodyn. Ther. 1 (2004) 345-346.

5. G.B. Rodrigues, L.K.S. Ferreira, M. Wainwright, G.U.L. Braga, Susceptibilities of the dermatophytes *Trichophyton mentagrophytes and T. rubrum* microconidia to photodynamic antimicrobial chemotherapy with novel phenothiazinium photosensitizers and red light, J. Photochem. Photobiol. B, 116 (2012) 89-94.

6. G.B. Rodrigues, M. Dias-Baruffi, N. Holman, M. Wainwright, G.U.L. Braga, *In vitro* photodynamic inactivation of *Candida* species and mouse fibroblasts with phenothiazinium photosensitisers and red light, Photodiag. Photodyn. Ther. 10 (2013) 141-149.

7. L. Ma, L.H. Xiang, B. Yu, R. Yin, L. Chen, Y. Wu, Z.J. Tan, Y.B. Liu, H.Q. Tian, H.Z. Li, T. Lin, X.L. Wang, Y.H. Li, W.Z. Wang, H.L. Yang, W. Lai, Low-dose topical 5-aminolevulinic acid photodynamic therapy in the treatment of different severity of acne vulgaris, Photodiag. Photodyn. Ther. 10 (2013) 583-590.

8. F. Pereira Gonzales, T. Maisch, Photodynamic inactivation for controlling *Candida albicans* infections, Fungal Biol. 116 (2012) 1-10.

9. A.K. Gupta, M. Paquet, F.C. Simpson, Therapies for the treatment of onychomycosis, Clin. Dermatol. 31 (2013) 544-554.

10. J.P. Tardivo, M. Wainwright, M.S. Baptista, Local clinical phototreatment of herpes infection in São Paulo, Photodiag. Photodyn. Ther. 9 (2012) 118-121.