Successful treatment of Pityriasis Versicolor by Photodynamic Therapy mediated by methylene blue

Journal:	<i>Photodermatology, Photoimmunology & Photomedicine</i>
Manuscript ID	PHOTO-OA-07-19-1168.R3
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Alberdi, Enrique Gómez, Clara; Spanish National Research Council, CSIC,
Keywords:	photodynamic therapy, methylene blue, Pityriasis versicolor

SCHOLARONE™
Manuscripts

Title: Successful treatment of *Pityriasis Versicolor* by Photodynamic Therapy mediated by methylene blue

Running Title: Treatment of *Pityriasis Versicolor* with PDT

Authors: Enrique Alberdi¹, Clara Gómez^{2*}

1 Private clinic of Dr. Alberdi, Aviador Zorita 25, 28020 Madrid

2 Institute of Physical Chemistry Rocasolano, CSIC, Serrano 119, 28006 Madrid

Address of authors:

Clara Gómez (author for correspondence)*:
Institute of Physical Chemistry Rocasolano
Spanish National Research Council, CSIC
C/Serrano 119, 28006 Madrid
Phone number: +34 91 7459532
Fax: + 91 5642431
c.gomez@iqfr.csic.es

Acknowledgements

This research was supported by a grant from the Eugenio Rodríguez Pascual Foundation (Madrid, Spain) and by the Spanish Research Projects MINECO (Ref.: MAT 2017-83856-C3). Authors wish to thank Dr. MJ Ortiz García for her input and suggestions while designing the study protocol.

Conflict of interest

The authors have no other relevant affiliation or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Abstract

Background: Although systemic therapies are recommended for severe or recalcitrant cases of Pityriasis versicolor (PV), they are not free of important side effects and drug interactions. Photodynamic therapy (PDT) utilizes the action of singlet oxygen and free radicals produced by a light-activated photosensitizer to kill viruses, bacteria or fungi. In this study the effect of a PDT mediated by methylene blue (MB) in PV was evaluated.

Methods: Five women with PV disseminated on the back and diagnosed by fresh microscopic analysis, were treated with a solution of MB (2%) applied to the PV lesions for 3 minutes. Next, a red LED lamp ($\lambda = 630 \pm 5$ nm, 37 J/cm^2), placed 100 mm from the skin for 10 minutes, was applied on the dyed PV lesions. Six sessions of MB/PDT were implemented with a 2-week interval in between. Wood's lamp examination was used to monitor fungal infection at each time point.

Results: Complete cure was observed in the five women at the 4-weeks post-treatment follow-up. Fluoresce images from PV lesions by Wood's lamp allowed to evaluate if the lesions were healed or not at each time point. No patient showed relapse at the 6-month follow-up. The patients did not have any adverse effect and good cosmetic outcome was observed.

Conclusions: Six sessions of MB/PDT spaced at 14-day intervals are sufficient for the treatment for PV in healthy patients.

Keywords: pityriasis versicolor, photodynamic therapy, methylene blue

SUMMARY STATEMENT

This is the first study where MB has been used as PS to carry out PDT for PV treatment.

This new PDT strategy is efficient, safe and inexpensive and would allow avoiding the severe side effects of the oral systemic drugs that usually are used for severe PV.

For Review Only

INTRODUCTION

Superficial mycoses have increased markedly over the last 20 years. They affect more than 25% of the world's population and represent one of the most frequent forms of infections [1, 2]. Among these kind of fungal infection is the Pityriasis versicolor (PV) (also known as tinea versicolor), which is caused by *Malassezia* spp. and often manifests during or after puberty. Hyperhidrosis is the most important predisposing factor; other risk factors include endocrinopathies, oral contraceptives, depressed cellular immunity, and the application of oily preparations [3, 4]. PV is clinically characterized by hypo- or hyperpigmented irregular macules and patches with fine scale, distributed over the neck, upper trunk, and proximal upper extremities [5, 6]. Widespread involvement of the lower extremities or face should raise concern for an immunosuppressive state such as HIV [7].

PV is managed primarily with topical agents and sun protection. The most effective topical agents are imidazoles and zinc pyrithione shampoos [8]. Effective oral regimes include itraconazole 200 mg daily for 5 to 7 days, fluconazole 300 mg per week for 2 weeks, and pramiconazole 200 mg daily for 2 days [8, 9]. Unfortunately, treatment failure of PV is common due to the long treatment duration, important side effects of the systemic antifungals, numerous interactions with other drugs and antifungal resistance [10]. Alternative antifungal strategies will be the key to the future treatment [11].

Photodynamic therapy (PDT) utilizes singlet oxygen and free radicals produced by a light-activated photosensitizer to kill virus, bacteria and fungus. The photochemical process is initiated by a low-power laser/light with an appropriate wavelength that is able to excite the photosensitizer (PS) and trigger photochemical reactions that generate

1
2
3 reactive oxygen species (ROS), such as singlet oxygen ($^1\text{O}_2$) and radicals. All these
4
5 species are cytotoxic, mainly singlet oxygen, thereby inducing microbial killing [12,
6
7 13]. The principles of photosensitization, specifically in the case of fungi, and some
8
9 clinical observations of PDT for treating superficial mycoses have been reviewed over
10
11 the last years [14, 15].
12
13
14

15
16 To the best of our knowledge, there have been no clinical trials of PDT against PV
17
18 except for one case where 5-aminolevulinic acid (ALA) was reported being used as PS
19
20 [16] for two sessions showing a good efficacy, but needing long incubation time and
21
22 covering with an adhesive occlusive dressing of polyurethane film. The aim of the
23
24 present work was to study for first time the efficacy of PDT mediated by methylene
25
26 blue (MB) as PS to treat and manage five cases of PV.
27
28
29

30
31 **MATERIALS AND METHODS**
32

33
34 **Patients**
35

36
37 A pilot trial of ~6-months duration was designed to achieve the proposed aims. Patients
38
39 with PV disseminated on the upper and lower back or shoulder were selected after
40
41 clinical (by dermoscopic images and by fluorescence from PV lesions) and mycological
42
43 diagnosis (by microscopic examination of scales soaked in KOH or by biopsy - only if it
44
45 was required-). Exclusion criteria were: age under 18 years, contraindications for the
46
47 use of MB (glucose-6-phosphate dehydrogenase deficiency), anti-depressant treatment
48
49 with MAO inhibitors, pregnancy or breastfeeding, use of antifungal shampoo, topical or
50
51 systemic antifungal agents within 0 to 24 weeks prior enrollment in the trial,
52
53 concomitants illnesses which could prevent the completion of the study, contact
54
55 dermatitis, atopic dermatitis, psoriasis, or any other skin disease. The study protocol
56
57 was approved by the institutional research ethics committee (Comité de Ensayos
58
59
60

1
2
3 Clínicos del Hospital Universitario San Carlos, Madrid, Spain; dated January 2018;
4
5 internal code: 17/501-E) in accordance with the declared ethical principles (World
6
7 Medical Association Declaration of Helsinki, 2008) [17], and written informed consent
8
9 was obtained from all subjects.
10
11
12

13 **Treatment**

14
15 Based on the protocol described by Souza and cols. and successfully tested by us for
16
17 onychomycosis treatment [18, 19], a sterile gauze was soaked in an aqueous solution of
18
19 MB (2%) and was then applied over PV lesions until the complete area of the lesions
20
21 was covered. Lesions were impregnated during 3 minutes (incubation time), without the
22
23 needed of occlusion treatment (Figure 1). Then the lesions were irradiated with light
24
25 from a LED lamp (Aktilite®, CL128) which emits red light with a narrow spectrum at
26
27 630 ± 5 nm, 37 J/cm^2 during 10 min. The lamp should be placed at 100 mm from the skin
28
29 and the area illuminated is 80×180 mm. For that reason, the lamp was moved as many
30
31 times as necessary to cover and irradiate the entire surface affected by PV, being
32
33 between 40 and 50 min. the total time taken by each session. Patient received 6 sessions
34
35 separated by an interval of 2 weeks.
36
37
38
39
40

41 A schematic picture of the study protocol is showed in Figure 2.
42
43

44 **Efficacy assessment**

45
46 Clinical efficacy assessment was done by observing three clinical signs and symptoms,
47
48 i.e. pruritus, hypo or hyperpigmentation, desquamation, and by dermoscopic images
49
50 taken at baseline and at each follow-up. In addition, detection of the fluorescence from
51
52 PV lesions after illumination with UV light by Wood's lamp was used for **monitoring**
53
54 the healing process and effectiveness of the MB/PDT. Safety assessment was also done.
55
56
57
58
59 Patients were considered as having clinical cure when dermoscopic images did not
60

show any of the proper characteristics of PV such as diffuse hypopigmented blotches (fairly demarcated) with fine scales.

Mycological efficacy assessment was carried out by direct microscopic analysis by KOH. Mycological cure was considered for negative KOH or absence of fungal hyphae or spores.

Complete cure was considered in patients with clinical as well as mycological cure.

At 4 weeks post-treatment and at 22-week follow-up, clinical and mycological examination was carried out.

RESULTS

After the application of restrictive inclusion and exclusion criteria and taking into account dropouts and exclusion patients with incompletely recorded histories, the final study population consisted of 5 systemically healthy women (ages from 33 to 59; mean age 43.2 ± 10.7 years old). Within this population, four women were diagnosed through KOH exam and only one skin biopsy was performed as there was some uncertainty about the diagnosis.

Figure 3 shows as an example of the clearance and healing process in two of the five patients. Wood's lamp examination allowed monitoring the healing process, being the yellowish-white fluorescence negative in 100% of the patients at the end of the treatment (10 weeks from the start of the treatment). Complete cure (clinical cure (by dermoscopic images) and mycological cure (KOH negative) was observed in all the patients at the 4 weeks post-treatment. At this time point, although the fine scales visible by scratching disappeared and PV lesions did not fluoresce when illuminated with the Wood's lamp, the pigmentary changes (in the five cases hypopigmentation)

1
2
3 **remained longer.** Relapse was not seen in any patient in the ~ 6-month follow-up. The
4
5 patients did not have any adverse effect or pain and good cosmetic outcome and
6
7 therapeutic outcome was observed.
8
9

10 11 12 13 14 **DISCUSSION**

15
16
17 It is very difficult to find an effective treatment of PV with systemic oral antifungal
18
19 without both important collateral side-effects and high risk of recurrence. Recently,
20
21 Romero-Sandoval and cols have studied and evaluated different therapeutic schemes
22
23 based in systemic antifungals for the treatment of recurrent and disseminated PV but
24
25 they found that most patients did not heal, which supports the chronic and refractory
26
27 nature of this clinical entity [20]. They hypothesized that recurrent and disseminated PV
28
29 depends on cellular or humoral balance, involving innate or acquired immune response
30
31 [20].
32
33
34
35

36
37 In real practice office, the correct treatment is prescribed based on patient age, medical
38
39 condition, length of therapy and likelihood of compliance, potential drug interaction,
40
41 cost of therapy, patient and physician preferences. Although guidelines recommend for
42
43 PV oral itraconazole for a long period of time, the patients of the present study chose an
44
45 alternative treatment based in PDT instead of the systemic oral one in order to avoid
46
47 systemic side-effects.
48
49

50
51 It is important to point out that PV is a chronically recurring fungal infection of the
52
53 stratum corneum (SC). The hydrophilic characteristics of the MB make it remain
54
55 confined in the SC after application on skin, where it absorbs light and exerts its action.
56
57 The choice of MB is very sensible for the treatment of PV. The phenothiazinium PSs,
58
59
60

such as toluidene blue TBO and MB are known to localize in the fungal membrane [21]. Consequently, this is the cellular structure damaged upon light exposure and it has been proposed that the increased permeability resulting from such damage is the reason for cell death [21, 22]. PDT as antifungal treatment option is cost-effective, highly selective and avoids the occurrence of drug-resistant strains [22]. MB as PS strongly absorbs light in the therapeutic window and it has a well-characterized and effective photochemistry that triggers both photosensitization mechanisms type I and type II. Increase in research on PDT mediated by MB is showing how this new PDT strategy is an efficient and inexpensive option [23, 24]. Its effects on yeasts and dermatophytes have led to the suggestion of its potential use for the treatment of skin mycoses [19, 22]. Therefore, MB/PDT may become a valuable alternative to the already established antifungal drugs, reinforced by the proven advantages of MB use as PS. In this regard, this is the first study where MB has been successfully used as PS to carry out PDT for PV treatment.

Although in the present study we describe a photodynamic action mediated by MB to treat PV, direct toxicity of MB to fungi has recently been pointed out specifically against human *Candida albicans* through dysfunctioning of mitochondria, disturbing redox status and disruption of membrane integrity leading to depletion in ergosterol level [25, 26]. Furthermore, MB inhibits the yeast to hyphal transition in *C. albicans* which is essential for survival and for virulence in the host body. MB can be taken into account as antifungal agent to treat *Candida* infections [25, 26]. Considering these conclusions, it would be interesting to analyze the contribution of MB *per se* and when MB is combined with the appropriate light, in the global anti-fungal effect against PV here obtained. To compare both contributions, the same concentration and incubation time would have to be set.

1
2
3 Mycological examination is considered to be the most important factor in determining
4 the efficacy of treatment. In the specific case of PV eradication, the mycological cure
5 rate was always higher than the clinical cure rate in all study groups. This implies that
6 part of the patients which were cured kept showing residual colour changes (mostly
7 hypopigmentation) [27]. Thus, patients must be reminded that pigmentary changes may
8 take weeks or months to clear, even if the fungus is eradicated.
9

10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
PV is a commonly recurrent superficial fungal infection of the skin. Patients need
effective follow-up care to implement a relapse prevention strategy. Due to the easy
availability of MB and its low cost, MB/PDT could also be tested as a prophylactic
treatment.

In conclusion, after 6 sessions of PDT/MB spaced by 14-day intervals, a prompt
clearance was observed without any recurrence signs 6 months after the start of the
treatment, confirming that it is a successful treatment in non-immunocompromised
patients with PV. More extensive studies are needed to corroborate the efficacy of the
present treatment, which is not associated with any of the important side-effects of
standardized systemic antifungals, and its capacity as prophylactic treatment when it is
used as maintenance therapy to avoid any recurrence.

References

- [1] Pedrosa AF, Lisboa C, Faria-Ramos I, Silva RM, Miranda IM, Rodrigues AG. Malassezia species retrieved from skin with pityriasis versicolor, seborrheic dermatitis and skin free of lesions: a comparison of two sampling methods. *Br J Dermatol*. 2018; 179(2): 526-527.
- [2] Pedrosa AF, Lisboa C, Rodrigues AG. Malassezia infections: a medical conundrum. *J Am Acad Dermatol*. 2014; 71(1):170-176.
- [3] Thoma W, Krämer HJ, Mayser P. Pityriasis versicolor alba. *J Eur Acad Dermatol Venereol*. 2005; 19 (2):147-152.
- [4] Hald M, Arendrup MC, Svejgaard EL, et al. Evidence-based Danish guidelines for the treatment of Malassezia-related skin diseases. *Acta Derm Venereol*. 2015; 95 (1):12–19.
- [5] Mathur M, Acharya P, Karki A, Kc N, Shah J. Dermoscopic pattern of pityriasis versicolor. *Clin Cosmet Investig Dermatol*. 2019; 12: 303-309.
- [6] Gupta AK, Bluhm R, Summerbell R. Pityriasis versicolor. *J Eur Acad Dermatol Venereol*. 2002; 16(1): 19-33.
- [7] Aly R, Berger T. Common superficial fungal infections in patients with AIDS. *Clin Infect Dis*. 1996; Suppl 2:S128-132.
- [8] Hu SW, Bigby M. Pityriasis versicolor: a systematic review of interventions. *Arch Dermatol*. 2010; 146(10):1132-1140.
- [9] Gupta AK, Lane D, Paquet M. Systematic review of systemic treatments for tinea versicolor and evidence-based dosing regimen recommendations. *J Cutan Med Surg*. 2014; 18(2):79-90.
- [10] Prohic A, Jovovic Sadikovic T, Krupalija-Fazlic M, Kuskunovic-Vlahovljak S. Malassezia species in healthy skin in dermatological conditions. *Int J Dermatol*. 2016; 55(5):494-504.
- [11] Wiederhold NP. Antifungal resistance: current trends and future strategies to combat. *Infect Drug Resist*. 2017; 10: 249-259.
- [12] Morton CA, Brown SB, Collins S, et al. Guidelines for topical photodynamic therapy: report of a workshop of the British Photodermatology Group. *Br J Dermatol*. 2002; 146(4): 552-567.
- [13] Wan MT, Lin JY. Current evidence and applications of photodynamic therapy in dermatology. *Clin Cosmet Investig Dermatol*. 2014; 7: 145-163.
- [14] Baltazar LM, Ray A, Santos DA, Cisalpino PS, Friedman AJ, Nosanchuk JD. Antimicrobial photodynamic therapy: an effective alternative approach to control fungal infections. *Front Microbiol*. 2015; 6: 202.

- [15] Donnelly RF, McCarron PA, Tunney MM. Antifungal photodynamic therapy. *Microbiol Res.* 2008; 163(1): 1-12.
- [16] Kim YJ, Kim YC. Successful treatment of Pityriasis Versicolor with 5-Aminolevulinic and Photodynamic therapy. *Arch Dermatol.* 2007; 143(9):1218-1220.
- [17] World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. World Medical Association. *JAMA.* 2013; 310(20): 2191-2194.
- [18] Figueiredo Souza LW, Souza SV, Botelho AC. Randomized controlled trial comparing photodynamic therapy based on methylene blue dye and fluconazole for toenail onychomycosis. *Dermatol. Ther.* 27(1): 43-47 (2014).
- [19] Alberdi E, Gómez C. Efficiency of methylene blue-mediated photodynamic therapy vs intense pulsed light in the treatment of onychomycosis in the toenails. *Photodermatol Photoimmunol Photomed.* 2019; 35(2):69-77.
- [20] Romero-Sandoval K, Costa AA, Teixeira Sousa MG, et al. Recurrent and disseminated pityriasis versicolor: A novel clinical form consequent to *Malassezia*-host interaction? *Med Hypotheses.* 2017; 109:139-144.
- [21] Bacellar IO, Pavani C, Sales EM, Itri R, Wainwright M, Baptista MS. Membrane damage efficiency of phenothiazinium photosensitizers. *Photochem Photobiol.* 2014; 90(4): 801-801.
- [22] Calzavara-Pinton PG, Venturini M, Sala R. A comprehensive overview of photodynamic therapy in the treatment of superficial fungal infections of the skin. *J Photochen Photobiol B*, 2005; 78(1):1-6.
- [23] Tardivo JP, Del Giglio A, de Oliveira CS, et al. Methylene blue in photodynamic therapy: From basic mechanisms to clinical applications. *Photodiagnosis Photodyn Ther.* 2005; 2(3):175-191.
- [24] Tardivo JP, Adami F, Correa JA, Pinhal MA, Baptista MS. A clinical trial testing the efficacy of PDT in preventing amputation in diabetic patients. *Photodiagnosis Photodyn Ther.* 2014; 11(3): 342-350.
- [25] Ansari MA, Fatima Z, Hameed S. Antifungal action of methylene blue involves mitochondrial dysfunction and disruption of redox and membrane homeostasis in *C. Albicans*. *Open Microbiol J.* 2016; 10: 12-22.
- [26] Pal R, Ansari MA, Saibabu V, Das S, Fatima Z, Hameed S. Nonphotodynamic roles of methylene blue: display of distinct antimycobacterial and anticandidal mode of actions. *J Pathol.* 2018, ID 3759704 Doi: 10.1155/2018/3759704.
- [27] Cam VT, Van TN, Hau KT, et al. Efficacy of Azole Antifungal in Treatment of Pityriasis Versicolor. *Open Access Maced J Med Sci.* 2019; 7(2):272-274.

Figure captions

Figure 1.- Application and incubation of MB aqueous solution.

Figure 2.- Schematic diagram of the experimental study design, (w): week; (s): session; (*): **monitoring** of the healing process by Wood’s lamp (before session 2-6).

Figure 3.- Clearance and healing process, (A) baseline, (B) delineated PV lesions by Wood’s lamp before PDT, (C) 22-week follow-up, (D) absence of PV lesions tested by Wood’s lamp at 22-week follow-up.

For Review Only

Figure 1

Application and incubation of MB aqueous solution.

Figure 2

Schematic diagram of the experimental study design, (w): week; (s): session; (*): monitoring of the healing process by Wood's lamp (before session 2-6)

Figure 3

Clearance and healing process, (A) baseline, (B) delineated PV lesions by Wood's lamp before PDT, (C) 22-week follow-up, (D) absence of PV lesions tested by Wood's lamp at 22-week follow-up.