

# Photodynamic Antifungal Therapy Against Chromoblastomycosis

Juliana Pereira Lyon · Conceição de Maria Pedroso e Silva Azevedo ·  
Leonardo Marmo Moreira · Carlos José de Lima ·  
Maria Aparecida de Resende

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**Abstract** Photodynamic therapy (PDT) is a minimally invasive approach, in which a photosensitizer compound is activated by exposure to light. The activation of the sensitizer drug results in several chemical reactions, such as the production of reactive oxygen species and other reactive molecules, which 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 PDT. This treatment might consist in an alternative for the management of fungal infections. Antifungal photodynamic therapy has been successfully employed against *Candida* species, dermatophytes, and *Aspergillus niger*. Chromoblastomycosis is an infection that involves skin and subcutaneous tissues caused by the traumatic inoculation of dematiaceous fungi species, being that the most prevalent are *Fonsecaea pedrosoi* and *Claphialophora carrionii*. In the present work, the clinical applications of PDT for the treatment of chromoblastomycosis are evaluated. We have employed methylene blue as photosensitizer and a LED (Light Emitting Diode) device as light source. The results of this treatment are positive, denoting the efficacy of PDT against chromoblastomycosis. Considering that great part of the published works are focused on in vitro trials, these clinical tests can be considered a relevant source of information about antifungal PDT, since its results have demonstrated to be promising. The perspectives of this kind of treatment are analyzed in agreement with the recent literature involving antifungal PDT.

J. P. Lyon (✉)

Departamento de Ciências Naturais, Universidade Federal de São João Del Rei. Praça Dom Helvécio, 74, Fábricas, 36301160 São João Del Rei, Minas Gerais, Brazil  
e-mail: julianalyon@yahoo.com.br

C. de Maria Pedroso e Silva Azevedo

Departamento de Medicina I, Centro de Ciências da Saúde, Universidade Federal do Maranhão. Rua Barão de Itapary, 227 – Hospital Presidente Dutra, 4º andar Centro, 65020-070 São Luis, Maranhão, Brazil

L. M. Moreira

Universidade Federal de São João Del Rei. Praça Dom Helvécio, 74, Fábricas, 36301160 São João Del Rei, Minas Gerais, Brazil

C. J. de Lima

Universidade Camilo Castelo Branco, Rodovia Presidente Dutra, Km 138, Parque tecnológico de São José dos Campos, São José dos Campos, São Paulo, Brazil

M. A. de Resende

Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Microbiologia. Av. Antonio Carlos, 6627, Pampulha, 31270901 Belo Horizonte, Minas gerais, Brazil

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## Introduction

Photodynamic therapy (PDT) is a treatment that employs a photosensitizer compound, which is activated by exposure to light of a wavelength that is excitatory to this compound. The activation of the sensitizer drug results in several chemical reactions, such as the production of reactive oxygen species (type II reaction) and other reactive molecules (free radicals, type I reaction). The presence of these molecules in the site to be treated leads to the damage of target cells [1]. PDT was firstly developed for the treatment of cancer, but recently it has been employed against several microorganisms, including bacteria, virus, and fungi [2–13]. Antifungal photodynamic therapy has been successfully employed against *Candida* species, dermatophytes, and *A. niger* [14–20].

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 restrict 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 [21]. Qiao et al. [22] developed an interesting review focusing on in vivo PDT for the treatment of superficial mycoses; however, the majority of the literature in this area concerns to in vitro experiments. Considering the potential of this technique in the treatment of fungal infections and the importance of developing new antifungal strategies, PDT can be considered an alternative of great interest to future researches.

Chromoblastomycosis is an infection that involves skin and subcutaneous tissues caused by the traumatic inoculation of a specific group of dematiaceous fungi. Several species have been implicated as etiological agents; however, the most prevalent in endemic areas are *F. pedrosoi* and *C. carrionii*. *F. compacta*, *P. verrucosa*, *R. aquaspersa*, or *E. dermatitidis* may also be causative agents of the disease [23]. These saprophyte fungi habit the soil and decayed materials, such as woods and leaves, which can explain the greater incidence of the disease in farm laborers.

The treatment of chromoblastomycosis can be performed by surgical removal of the lesions,

cryosurgery, employment of antifungal agents, or a combination of these treatments. However, it is important to register that the complete healing is difficult and recurrences are common [23]. The administration of antifungal agents for long-term treatments can cause several undesirable side effects and toxicity to the patient, and the surgical treatment can be mutilating and painful. Considering these facts, the application of PDT for the treatment of chromoblastomycosis is an issue of interest, especially in recurrent fungal infections, even if employed as adjuvant to conventional antifungal therapy. The procedure is safe and does not cause additional damage to the patients. In the present work, 10 patients with chromoblastomycosis were treated with PDT, employing methylene blue as photosensitizer and a LED (Light Emitting Diode) device as light source.

## Materials and Methods

### Patients

Ten patients infected with chromoblastomycosis were selected from the Ambulatory of Chromoblastomycosis in the Infectious/Parasitic Diseases Service of the Federal University of Maranhão (UFMA), São Luís, Maranhão, Brazil. The patients selected did not receive antifungal treatment in the past 6 months before the treatment and did not presented any kind of additional disease or predisposing condition for other infections. The chromoblastomycosis infection was diagnosed by a dermatologist through physical examination, direct observation of fungal structures in the microscope, cultivation, and direct observation of the fungus in the lesion. No particular stage of the disease was used as selection criterion. The protocol of the study was approved by the Ethics Committee of the institution, and a consent formulary was signed by each patient.

### Antifungal Photodynamic Therapy

The photosensitizer delivery was carried out as described by Calzavara-Pinton et al. [18] with modifications. A 20% methylene blue preparation in Eucerin cream (Beiersdorf, Hamburg, Germany) was applied to the skin lesions for 4 h under a gauze occlusive dressing, which was removed prior to the irradiation. After this period, the lesions were

irradiated with red LED (GaAlAs, wavelength absorption 660 nm, energy dose of 28 J/cm<sup>2</sup>) for 15 min. If the lesion extension exceeded the LED reaching area, the irradiation was repeated in the same patient until all the compromised area to be treated. The light intensity was uniform in all the areas treated, and the treated areas varied between the patients. Any undesirable side effect was registered during the treatment.

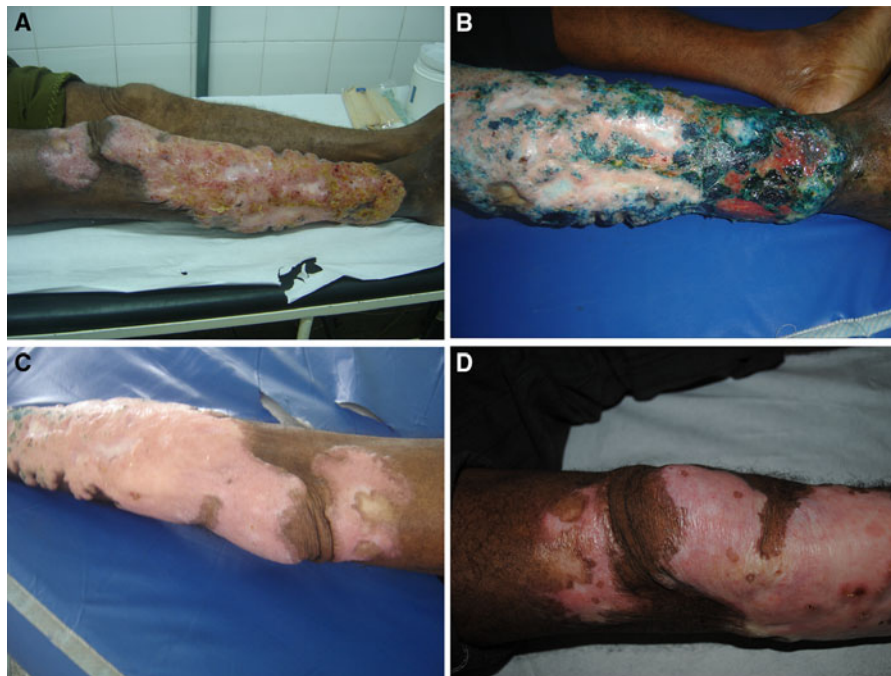
The lesions were clinically and microscopically observed after 7 days. The treatment was repeated for five additional weeks. The lesions were recorded for comparison before and after the treatment. Patients returned after 1 week for follow-up and for treatment with PDT. The total follow-up period is 2 years after the complete remission of the disease and negative mycological tests.

## Results and Discussion

All of the ten patients treated presented reduction in the compromised area after six PDT applications, considering clinical and microscopical aspects. The

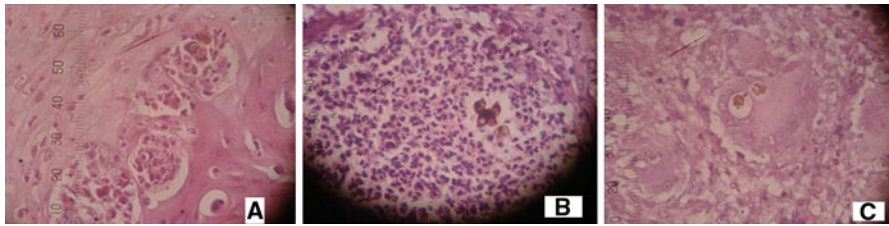
reduction was measured as about 80–90% of improvement in the aspect of the lesion, i.e., reduction in the lesion volume and tissue cicatrization in 80–90% when compared with the previous condition of the lesion.

Figure 1 corresponds to the evolution of the healing in one of the patients treated. The efficacy of this alternative treatment was verified by expertise doctors and confirmed by biopsy. The treatment was well tolerated by all patients evaluated, and no side effects were registered. Patients reported that the pain sensation diminished, as well as the disappearance of the odor and rapid reduction in the lesions. They also felt hopeful and excited about the treatment. Patients with severe lesions reported that they were less worried about the aspect of their lesions. However, the complete healing was not achieved in any patient, and the mycological tests were still positive, except for one patient. These positive tests showed the necessity of adjutant treatment with itraconazole after the period of treatment with PDT. Figure 2 shows histopathological images of the lesions. The use of PDT as a coadjutant treatment does not make this



**Fig. 1** Clinical aspect of chromoblastomycosis lesion in one patient, before and after antifungal photodynamic therapy. **a** Initial aspect of the lesion before treatment. **b** Aspect of the

lesion after 1 week of treatment. *Methylene Blue* was applied in the skin. **c** Aspect of the lesion after 4 weeks of follow-up. **d** Aspect of the lesion after 1 additional week of follow-up



**Fig. 2** Classic histopathological images of chromoblastomycosis infection observed in patients before the photodynamic treatment. **a** Microabscesses. **b** Muriform cells. **c** Giant cell

therapy irrelevant, since it can reduce the size of the compromised areas and the degree of infection. This can lead to an improvement in the efficacy of the antifungal chemotherapy, as well as in reduction in the time of antifungal administration, less toxicity to the patient, and eventually in diminishing the relapses cases. Furthermore, the quality of life of the patients is significantly improved during the therapy, decreasing the number of patients that abandon the treatment.

The results obtained in this clinical trial do not allow the elucidation of the mechanism involved in the healing process. However, we could infer that PDT may act by paralyzing the growth, killing the dematiaceous fungi, or even by interfering in the immunologic mechanism. In any case, additional studies are necessary to clarify the particular action of PDT against chromoblastomycosis infections.

Despite this preliminary results obtained with methylene blue do not achieve the complete healing, it indicates promising applications for PDT in chromoblastomycosis. Other photosensitizer agents as well as other therapeutic schemes might be tested to obtain better results.

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