Photodynamic inhibition of *Trichophyton rubrum*: in vitro activity and the role of oxidative and nitrosative bursts in fungal death

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Objectives: Antimicrobial photodynamic inhibition (aPI) is based on the use of a light source and a photosensitizer to kill pathogens. Little is known about aPI of dermatophytic fungi and its mechanism of action. We aimed to evaluate aPI of *Trichophyton rubrum*.

Methods: We performed tests using toluidine blue (TBO) as a photosensitizer and a 630 nm light-emitting diode (LED) as a source of light to target 12 *T. rubrum* isolates. Susceptibility testing with cyclopiroxolamine, time–kill curves and quantification of reactive oxygen species (ROS), peroxynitrite (ONOO⁻) and nitric oxide (NO⁻) were performed.

Results: The optimal conditions for in vitro aPI were 10 mg/L for TBO and 48 J/cm² for LED; these conditions were fungicidal or inhibited ≥98% of fungal growth depending on the strain tested. LED or TBO treatment alone did not inhibit growth. The MICs of cyclopiroxolamine were 2.0 mg/L for 90% of the strains. Analysis of time–kill curves revealed that pathogen death occurred 24 h post-treatment. Quantification of ROS, ONOO⁻ and NO⁻ revealed improvement after aPI.

Conclusions: Photodynamic inhibition was more efficient in promoting cell death than the antifungal cyclopiroxolamine against *T. rubrum*. ROS, ONOO⁻ and NO⁻ were important in the fungicidal activity of aPI. A suggested mechanism for this activity is that TBO is excited by LED light (630 nm), reacts with biomolecules and increases the availability of transition electrons and substrates for nitric oxide synthase, thereby increasing the oxidative and nitrosative bursts in the fungal cell.

Keywords: antimicrobial photodynamic treatment, dermatophytes, LED, TBO, cyclopiroxolamine

Introduction

Dermatophytosis is a superficial infection caused by dermatophytes, a group of keratinophilic fungi that infect keratinized tissues such as nails, skin and hair.¹ This disease occurs in immunocompetent and immunocompromised individuals. *Trichophyton rubrum* is the most common causative agent of onychomycosis (fungal nail infections), a dermatophytosis of high incidence, and its rapid evolution may be regarded as a marker of immunodeficiency (e.g. HIV infection). In immunodeficient individuals onychomycosis caused by *T. rubrum* can involve all fingers and toenails.²,³ There are a few medications for the treatment of dermatophyte infections, including azoles (itraconazole), allylamines (terbinafine) and hydroxypyridone (cyclopiroxolamine).⁴,⁵ Cyclopiroxolamine exhibits broad-spectrum activity and can be used as a topical agent.⁵ Cyclopiroxolamine differs from other topical agents because it does not act by inhibiting the synthesis of ergosterol. Instead, it shows high affinity for trivalent metal cations, resulting in the inhibition of cytochrome oxidase and metal-dependent enzymes, which are responsible for the degradation of peroxides inside the fungal cell.⁴,⁷

Studies have shown that dermatophytoses caused by *T. rubrum* are often associated with treatment failure and relapses following cessation of treatment.⁸–¹¹ Treatment failure can be expected in 20%–30% of cases of onychomycosis.¹² The therapy can frequently be long-lasting and expensive for the patient, and treatment does not always cure the lesion.¹⁰,¹¹,¹³ The success of treating dermatophytosis is usually related to the ability of an antifungal drug to promote mycological cure, i.e. absence of clinical signs and the presence of negative nail culture, which denotes the
complete elimination of fungal cells from the host. In cases where there is resistance to antimicrobial drugs, arthroconidia and dormant spores are responsible for relapses of dermatophytoses. These drawbacks found during antimicrobial drug therapy usually lead to insufficient elimination of the fungus, which makes the study of other therapeutic strategies, such as antimicrobial photodynamic inhibition (aPI), of major prospective interest for effective clinical intervention in dermatophytosis.

aPI combines a photosensitizer (PS) and a light source to induce a phototoxic reaction that causes cell death. In most cases, molecular oxygen is required for the phototoxic reaction triggered by aPI to occur. During aPI, the light source emits at the appropriate wavelength, exciting the PS, which generates two types of reactions: type I and type II. The type I reaction, electron transfer between the PS and the biological molecules results in the production of harmful reactive intermediates such as superoxide, hydroperoxyl and hydroxyl radicals, and hydrogen peroxide (H₂O₂). The type II reaction results from an energy transfer that leads to excitation of the PS to a triplet state. This process results from an in situ electron spin exchange between the PS and molecular oxygen, resulting in the generation of an oxygen singlet, which is the main mediator of aPI.

Phenothiazines [toluidine blue (TBO) and methylene blue], phthalocyanines, halogenated xanthenes (rose Bengal) and porphyrines are chemical compounds with photoactive properties that have been used as the PS. Multiple studies have demonstrated the effectiveness of photodynamic inhibition as a fungicidal or bactericidal tactic, although not much is known about aPI and its mechanism of action against dermatophytic fungi. The aim of this study was to evaluate the activity of aPI of the dermatophyte T. rubrum. We also quantified the production of oxygen and nitrogen species during aPI and verified the susceptibility of T. rubrum to the topical antifungal cyclopiroxolamine. This drug was chosen because it is available in multiple formulations such as cream, nail lacquer, gel and shampoo.

**Materials and methods**

**T. rubrum isolates**

A set of 12 T. rubrum strains were selected for in vitro susceptibility testing with cyclopiroxolamine and photodynamic inhibition tests. One of these strains was reference strain ATCC 28189 (INCQS40051), and 11 of the strains were from clinical isolates from Belo Horizonte city, Minas Gerais, Brazil. All of the strains were maintained in sterile distilled water at 4°C, and were subcultured on potato dextrose agar (PDA) at 28°C for 7 days to produce conidia before the tests.

**Preparation of the inocula**

The inocula were prepared according to the protocol established by Santos and Hamdan. Seven-day-old colonies were covered with 5 mL of PBS. A mixture of conidial and hyphal fragments was filtered with a Whatman filter model 40 (pore size 8 μm), which retains hyphal fragments and only permits the passage of T. rubrum microconidia. Inocula were quantified by counting microconidia in a haemocytometer, with the aim of obtaining 1×10⁵ cells/mL to perform aPI and 4×10⁴ cells/mL to perform susceptibility testing with cyclopiroxolamine. The same concentrations of microconidia (1×10⁵ and 4×10⁴ cells/mL) were used for determination of time–kill curves after aPI and treatment with cyclopiroxolamine, respectively.

**Evaluation of susceptibility to cyclopiroxolamine**

All of the T. rubrum isolates were tested with cyclopiroxolamine (Pratti, Donaduzzi, São Paulo, Brazil) by a microdilution method according to previous studies. The standard RPMI 1640 medium (Himedia, Brazil) at 34.54 g/L was buffered with 0.165 M MOPS (Sigma-Aldrich, St Louis, MO, USA) and supplemented with 2% of glucose (Vetc, Brazil). The anti-fungal drug was dissolved in 100% DMSO (Synth) and prepared in a stock solution of 1000 mg/L. Serial 2-fold dilutions in RPMI were performed. The concentration range varied from 0.031 to 16.0 mg/L, and 96-well flat-bottomed microdilution plates were used. Each microdilution well containing 100 μL of the 2-fold drug concentration was inoculated with 100 μL of the inoculum suspension. For each test plate, two drug-free controls were included, one with the medium alone (sterile control) and the other with 100 μL of medium plus 100 μL of inoculum suspension (growth control). The microdilution plates were incubated at 28°C, and were read visually after 7 days of incubation. The MIC was visually determined as the concentration giving 80% inhibition of fungal growth compared with the control growth.

**Photosensitizer**

TBO (Sigma-Aldrich) was used as the PS at concentrations of 70 mg/L (0.15 mM), 40 mg/L (0.09 mM) and 10 mg/L (0.022 mM). A stock solution was prepared by diluting the TBO in sterile MilliQ water and storing it at 4°C in the dark.

**Light source**

A light-emitting diode (LED; Fisioled, MMoptics LTDA, São Carlos, SP, Brazil) was used as a source of monochromatic light. The red light was emitted at a wavelength of 630 nm (±10 nm, which is resonant to the TBO absorbance peak of 633 nm) at light doses of 18, 48 and 72 J/cm². The power output was 100 mW, the irradiance was 100 mW/cm², and the spot area of 1.0 cm² was produced in an active medium containing indium, gallium, aluminium and phosphorus (InGaAlP).

**Optimization of conditions for in vitro aPI of T. rubrum**

The T. rubrum strain ATCC 28189 (INCQS40051) was chosen for the standardization step, and the optimal conditions were applied to the other 11 strains. All tests were performed in sterile 96-well flat-bottom black microplates (Optiplate™, 96 Black, PerkinElmer, Waltham, MA, USA) with a maximum suspension volume of 200 μL. For determination of the optimal TBO concentration and LED light dose, the fungal conidia were divided into eight independent groups as described in Table 1. After these procedures, 10 μL of suspension was subcultured in Petri dishes with PDA and incubated at 28°C for 72 h until viable colonies could be counted.

**Susceptibility of the 12 T. rubrum strains to aPI**

Based on the results of the previous tests, the optimal conditions for performing in vitro aPI of T. rubrum were a TBO concentration of 10 mg/L and a light (630 nm) dose of 48 J/cm². These conditions were used to test 12 different T. rubrum isolates for their susceptibility to treatment. Growth control by aPI was calculated using the average growth for all of the controls of each isolate evaluated.
Table 1. Groups and conditions used for in vitro aPI for the determination of the optimal TBO concentration and LED light dose

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBO concentration&lt;sup&gt;a&lt;/sup&gt;</th>
<th>LED light dose&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>control groups</td>
<td>C1, untreated</td>
<td>C1, untreated</td>
</tr>
<tr>
<td></td>
<td>C2, exposed to TBO at 10 mg/L without light irradiation</td>
<td>C2, exposed to TBO at 10 mg/L without light irradiation</td>
</tr>
<tr>
<td></td>
<td>C3, exposed to TBO at 40 mg/L without LED irradiation</td>
<td>C3, exposed to TBO at 40 mg/L without LED irradiation</td>
</tr>
<tr>
<td></td>
<td>C4, exposed to TBO at 70 mg/L without LED irradiation</td>
<td>C4, exposed to TBO at 70 mg/L without LED irradiation</td>
</tr>
<tr>
<td>treatment groups</td>
<td>T1, exposed to TBO at 10 mg/L then LED irradiation at 72 J/cm²</td>
<td>T1, exposed to TBO at 10 mg/L then LED irradiation at 72 J/cm²</td>
</tr>
<tr>
<td></td>
<td>T2, exposed to TBO at 40 mg/L then LED irradiation at 72 J/cm²</td>
<td>T2, exposed to TBO at 40 mg/L then LED irradiation at 72 J/cm²</td>
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<tr>
<td></td>
<td>T3, exposed to TBO at 70 mg/L then LED irradiation at 72 J/cm²</td>
<td>T3, exposed to TBO at 70 mg/L then LED irradiation at 72 J/cm²</td>
</tr>
</tbody>
</table>

<sup>a</sup>min of pre-incubation of the fungal conidia with TBO was performed in the dark.

Time–kill curve

After treatment with aPI (10 mg/L of TBO and an LED light dose of 48 J/cm²) or with cyclopiroxolamine (at the MIC concentration), 100 µL of the suspension of strain ATCC 28189 was transferred to 96-well microdilution plates with 100 µL of RPMI 1640 medium (Himedia) at 34.53 g/L buffered with 0.165 M MOPS (Sigma-Aldrich) and supplemented with 2% glucose (Vetec). The microdilution plates were incubated at 28°C for 4 h with further removal of a 100 µL aliquot and addition of 100 µL of DMSO. Reading was performed at 490 nm in a spectrophotometer.

Evaluation of the effect of in vitro photodynamic inhibition on the oxidative and nitrosative bursts

The T. rubrum strain ATCC 28189 was used for evaluating the role of oxidative and nitrosative stress in fungal death using two different experimental strategies. In the first strategy (Protocol i), fungal cells were exposed to radicals without aPI: H₂O₂ (0.088 mM; 0.88 mM and 8.8 mM; Synth<sup>a</sup>) was used as a reactive oxygen species (ROS) producer, 3-morpholinosydnonimine (SIN-1) (0.01, 0.1 and 1 mM; Invitrogen) as a peroxynitrite (ONOO⁻) producer and sodium nitroprussiate (NPS) (0.01, 0.1 and 1 mM, Sigma-Aldrich) as a nitric oxide (NO⁻) producer. These distinct concentrations were added individually to the conidial suspension so that the levels of the radicals and their ability to reduce fungal viability could be evaluated. All samples were incubated for 2 h in RPMI 1640 without phenol red (Cultilab, Brazil) plus 2% glucose at 28°C. The optimal concentration (defined by fluorescence analyses as described below) of each radical producer (8.8 mM for H₂O₂, and 1 mM for each of SIN-1 and NPS) was also used when measuring fungal viability by counting the cfu after plating 100 µL in PDA and incubating at 28°C for 72 h. In the second strategy (Protocol ii), the radicals were removed from the suspension during in vitro photodynamic inhibition. At the moment of performing aPI, 20-tetrais-(4-sulfonatophenyl)-porphyrin iron (III) chloride (fetpps) (0.1, 1 and 10 µM, Merck) was used as an ONOO⁻ scavenger, 4,5-dihydroxy-1,3-benzenedisulfonic acid disodium salt hydrate (Tiron) (5, 50 and 500 µM, Sigma-Aldrich) was used as a superoxide anion scavenger and Nω-nitro-ω-arginine methyl ester hydrochloride (l-NAME) (0.05, 0.5 and 5 mM, Sigma-Aldrich) was used as a nitric oxide synthase (NOS) inhibitor. These drugs, diluted according to the manufacturers’ instructions, were individually diluted in PBS and added to the fungal suspensions at the start of the treatment. The samples were incubated for 2 h on RPMI 1640 without phenol red plus 2% glucose at 28°C. For both of the protocols (i and ii), the radical levels were measured by treating the sample with 10 µM of 4-amino-5-methylamino-2,7'-difluoro fluorescein (DAF-FM, Invitrogen) to quantify NO⁻; 50 µM of 2',7'dichlorodihydrofluorescein diacetate (H₂DCFDA, Invitrogen) was used to quantify ROS and 25 µM dihydrodorhamidine 123 (DHR 123, Invitrogen) was used to quantify ONOO⁻ for 30 min at 28°C. For all analyses, fluorescence was determined in a fluorimeter (Synergy 2, BioTek, USA) at a wavelength of 485 nm and a measuring emission wavelength of 530 nm. The data were expressed as the means ± SEM for the fold increase of fluorescence over the control. The optimal concentration of each scavenger and inhibitor (500 µM for Tiron, 10 µM for Fetpps and 5 mM for L-NAME) was also used when measuring fungal viability after aPI treatment by counting the cfu after plating 100 µL in PDA and incubating at 28°C for 72 h.

Data analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA), Kruskal–Wallis and Newman–Keuls multiple comparison tests. A P value of <0.05 was considered to be significant.

Results

Antifungal susceptibility testing with cyclopiroxolamine

The MIC values for T. rubrum varied from 1.0 to 16 mg/L. The MIC<sub>50</sub> and MIC<sub>90</sub> are the values that inhibited 50% and 90% of all tested isolates, respectively. In this study, these values were the same: 2.0 mg/L.

Establishing the optimal conditions for in vitro aPI against T. rubrum

For fungal suspensions containing the three different concentrations of TBO (70, 40 and 10 mg/L), the light (630 nm) dose at 72 J/cm² resulted in complete inhibition of T. rubrum ATCC 28189 growth on PDA (P < 0.0001) (Figure 1a and c). The TBO concentration of 10 mg/L was selected for further investigation of the optimal LED light dose. When distinct light (630 nm) doses (18, 48 and 72 J/cm²) were tested, we verified that the two higher doses (48 and 72 J/cm²) completely inhibited fungal growth on PDA (P < 0.0001) (Figure 1b). In all experiments, an LED light dose without TBO or TBO without an LED light dose did not reduce the cell viability of T. rubrum compared with the control group (Figure 1a and b). However, aPI efficiently decreased the number of viable colonies compared with the experimental controls (Figure 1a–c). The experimental conditions of 10 mg/L of TBO and 48 J/cm² of light (630 nm) were selected to test the other T. rubrum strains.
Effect of photodynamic inhibition on 12 isolates of \textit{T. rubrum}

Antimicrobial photodynamic inhibition (TBO 10 mg/L and LED light dose of 48 J/cm$^2$ at 630 nm) completely inhibited the growth of nine of the \textit{T. rubrum} strains (including ATCC 28189). Growth inhibition of 99\% (reduction of 1.7 log), 98\% (reduction of 1.6 log) and 94\% (reduction of 0.9 log) were obtained for strains 665, 916 and 281, respectively (Figure 1d). Considering all of the isolates tested, the average rate of inhibition was...
98%. Interestingly, the cyclopixrolamine MIC for strain 281 was 16 mg/L, this being the least susceptible isolate tested with this drug. These data correlate with the lower susceptibility of this strain to aPI (Figure 1d).

**Time–kill curve**

*Time–kill assays based on MTT reduction*

There was a 27% and 100% reduction in the viability of *T. rubrum* ATCC 28189 after 12 and 24 h, respectively (Figure 2) for cyclopixrolamine. The reduction in viability was 4.3% after 12 h and 86.5% after 48 h, which was maintained until 96 h of incubation (Figure 2).

**Time–kill assays performed by counting cfu/mL**

The results corroborated those of MTT assays: using the same inoculum size and plating times it was observed that treatment by aPI reduced colony counts by 98%–100% (fungicidal effect) since time 0 h. Growth reduction by cyclopixrolamine was only recorded after 48 h of incubation, and reduced colony counts occurred in the range of 70%–85% only (fungistatic effect).

**Evaluation of the effect of in vitro photodynamic inhibition on the oxidative and nitrosative bursts**

When the *T. rubrum* conidia were exposed to producers of NO–(NPS), ROS (H₂O₂) and ONOO– (SIN-1), we observed a significant increase (*P*<0.0001) in radical levels (fluorescence) (Figure 3a–c) and a drastic reduction in fungal viability (Figure 3d), primarily after exposure to H₂O₂. However, when the scavengers of ONOO– (Fetpps) and superoxide anion radicals (Tiron) were used concomitantly with aPI treatment, we noted that the decrease in fluorescence occurred in a concentration-dependent manner when compared with the control (aPI without any scavenger or inhibitor) (*P*<0.0001), except for L-NAME (upstream inhibitor of NO–), which at the lowest concentration was already able to reduce NO– production. The highest concentrations of Fetpps and Tiron resulted in the lowest levels of fluorescence. Interestingly, Fetpps (10 μM), L-NAME (5 μM) and Tiron (500 μM) significantly reduced (*P*<0.05) the efficiency of aPI by increasing fungal viability (Figure 3e–h).

**Discussion**

There are few studies demonstrating the use of TBO and 630 nm red light emitted from an LED source for the inactivation of filamentous fungi, and our results showed the efficacy of aPI of *T. rubrum*. The optimal conditions for photodynamic inhibition were 10 mg/L of TBO and 48 J/cm² of LED (Figure 1a). These parameters caused 98% reduction in *T. rubrum* viability, and the time–kill curve revealed that the viability of the conidia was completely abolished after 24 h of treatment (Figure 2). Other studies reported a fungicidal effect on *T. rubrum* after in vitro aPI when using 5,10,15-Tris(4-methylpyridinium)-20-phenyl-[21H,23H]-porphine trichloride (Sylsens B) as the PS and a white lamp (dose of 1080 kJ/m²)³⁰ or when using 5-aminolevulinic acid (ALA) and a quartz–halogen lamp (dose of 128 J/cm²) as a light source.⁳⁹ LED light and TBO have also exhibited fungicidal activity against yeast species, as evidenced with *Candida albicans* and *Cryptococcus gattii*. Using an ex vivo model of the human stratum corneum, it was demonstrated that both hyphae and conidia of *T. rubrum* were susceptible to photodynamic inactivation.⁴⁰

LED light or TBO alone did not inhibit fungal growth, demonstrating that the resonance between the light source and PS is necessary to produce the deleterious effect of aPI. Within the three concentrations of TBO and three LED light doses tested, the combination of the smallest effective concentrations and doses was chosen to perform the other tests.

The cyclopixrolamine MIC₅₀ and MIC₉₀ determined for the 12 strains tested were higher than those demonstrated by Ghannoum et al.⁴¹,⁴² and de Assis Santos et al.⁴³ However, the first group performed susceptibility tests with a lower inoculum of both hyphae and conidia. In addition, this discrepancy might result from the difference in the set of strains used in the studies. Treatment with cyclopixrolamine was fungistatic after 96 h of observation since it was possible to detect viable cells, as indicated by the MTT assay and cfu counting (data not shown). This observation was different from what is seen with aPI, which was shown here to be fungicidal in vitro with an impressive reduction in fungal viability after a shorter period of time, as attested by the time–kill curves. This result suggests that aPI is more efficient in promoting cell death than the anti-fungal cyclopixrolamine and therefore may be an interesting option for treating dermatophytosis.

All of the radicals studied were able to kill *T. rubrum*, as demonstrated when NPS, H₂O₂ and SIN-1 were tested. Analysis of the oxidative and nitrosative bursts showed an increase in the levels of NO–, ROS and ONOO– after aPI when compared with the controls (*P*<0.0001). Upon light irradiation, PS promotes a photochemical reaction resulting in the production of ROS, mainly singlet oxygen (¹O₂) and O₂. These ROS species react with various cellular components, resulting in damage by lipid peroxidation and disruption of structural proteins, enzymes and
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nucleic acids. Soares and coworkers showed a substantial increase in the production of ONOO- after photodynamic inactivation of C. gattii. Our study showed that when oxidative and/or nitrosative stress is disturbed by an NOS inhibitor and scavengers of O2- and ONOO-, the production of these radicals was impaired and the efficiency of treatment was significantly compromised. The use of scavengers such as sodium azide (scavenger of O2-) and hydroxyl radicals (OH-) conferred protection and substantially reduced the effects of aPI in C. albicans and Escherichia coli B, respectively.

Furthermore, our data clearly demonstrate that aPI triggers the production of NO-, O2-, and ONOO- in the fungal cell, which appears to be critical for killing T. rubrum, demonstrating that both oxidative and nitrosative bursts are important. The production pathways of these radicals are poorly understood in fungi. Intriguingly, our results demonstrated that the considerable quantities of neutralizing enzymes in eukaryotic cells are not able to prevent the damage caused by the ROS and reactive nitrogen species (RNS) generated by aPI. The burst provided by aPI may lead to cell death by apoptosis or to death caused by damage to the lipid membrane, enzymes or nucleic acids.

The generation of NO- in eukaryotic cells occurs by the oxidation of L-arginine by NOS. NO- can react with superoxide anions, generating ONOO-, a powerful oxidant. This study pointed to the presence and the important role of the enzyme NOS in T. rubrum once L-NAME, an inhibitor upstream of NOS, reduced the content of NO- in the fungal cell. The generation of NO- and ONOO- after treatment may be related to the reaction of activated TBO with biomolecules in the cytosol, cell membrane or extracellular compounds, which leads to the increased availability of L-arginine. Beyond this function, TBO binds to the outer membrane of fungal cells during the pre-irradiation period and becomes activated after irradiation, increasing its cytosolic penetration and its reactions with biomolecules.

The results obtained following quantification of the superoxide anion supported the hypothesis that cellular death after aPI is efficiently caused by damage to the lipid membrane, enzymes or nucleic acids.

In conclusion, the in vitro aPI of T. rubrum is more efficient in promoting cell death than the antifungal ciclopiroxolamine. Furthermore, our study demonstrated that T. rubrum is efficiently inactivated by aPI, suggesting that aPI would be a useful tool to treat the dermatophytosis caused by this fungus and probably other dermatophytes.

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Transparency declarations
None to declare.

References
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