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Action of photodynamic inactivation in antibiotic failures

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Significance: The increase in the number of microorganisms resistant to antimicrobials motivates the search for strategies that recover the use of traditional antibiotics. Photodynamic Inactivation (PDI) is an alternative to antibiotics because the combination of a photosensitizer with light leads to the production of reactive oxygen species that promote the elimination of undesirable cells.
Approach: Curcumin and light at 450 nm are applied at regular intervals and new values of minimum inhibitory concentration of antibiotics are obtained.
Results: We demonstrated that the application of PDI reduces the necessary dosage of antibiotics for the elimination of the bacterial cell, proving to be an efficient strategy for application in resistant strains. Furthermore, we verified that the interaction of the photosensitizer with the antibiotics promotes alterations in the metabolism and the bacterial biomolecules.
Conclusions: These results are essential to transfer in vitro studies to the clinical implementation of DIF as an adjuvant to antibiotic therapy.

Keywords: photodynamic inactivation, bacterial resistance, antibiotics

1. Introduction and Background

Resistant bacteria are one of the main health problems worldwide. The increase in the number of microorganisms resistant to antimicrobials motivates the search for strategies that recover the use of traditional antibiotics [1]. One strategy is the combination of therapies to combat or delay resistance to antimicrobials. In this scenario, photodynamic inactivation (PDI) is a technique with antimicrobial efficiency widely reported in the literature, in addition to the advantages of low side effects and mainly because its mechanism of action is based on bacterial death through oxidative stress, which is likely to development of previously unreported resistance [2], makes it an excellent adjunct to antibiotic therapy [3].

2. Aims

Evaluate the combination of PDI with antibiotics to enhance both techniques and ensure possible antagonistic or synergistic effects of drug interactions to ensure safe national implementation.

3. Methods

The minimum inhibitory concentration (MIC) for Staphylococcus aureus of the antibiotics amoxicillin, erythromycin, and gentamicin was determined as described by the Clinical and Laboratory Standards Institute (CLSI).
PDI protocols were applied, using curcumin as a photosensitizer (10 μM) and a light dose of 10 to 20 J/cm^2 using the Biotable ® (illumination device containing 24 uniformly distributed LEDs) at a wavelength of 450 nm. Each experiment has two groups, with three repetitions each: 1) only bacteria, bacteria with PS (10 μM), and bacteria irradiated (10 J/cm^2); 2) bacteria with PS irradiated. All samples were diluted and plated in Petri dishes with BHI agar medium and incubated at 37°C for 24 h.
The bacteria were first submitted to PDI and then transferred to cuvettes with the addition of antibiotics with concentrations close to the MIC, defined as time zero. The optical density at 600 nm of each group of the respective monotherapies was measured and the control group during 8 hours to verify the growth curve of the respective treatments.

4. Results

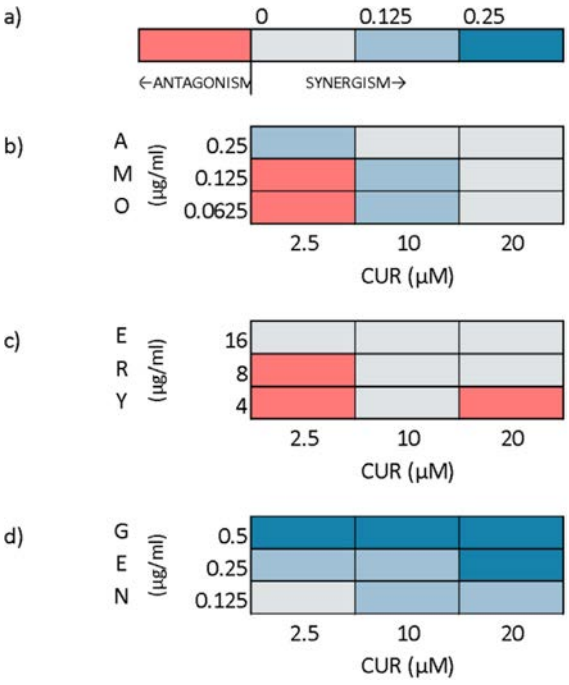

Figure 1 Evaluation of the synergistic or antagonistic effect of the Bliss independence model for DIF and ANTB. Synergy (S>0) is represented in blue scale and antagonism (S<0) is represented in red. A) Color scale. B) Group with Amoxicillin (AMO). C) Group with Erythromycin (ERI). D) Group with Gentamicin (GEN).

The combined effect of two treatments represented by the Bliss independence model is defined as the probability of the microorganism being affected by at least one of the treatments acting independently.35 In Figure 1, the synergistic combination by the Bliss independence model was observed in 81.5% of the combined groups (N=27), i.e., although the monotherapies used were underdose, the combinations resulted in promising bacterial inactivation. The antagonistic combinations were observed in the groups whose one of the monotherapies (antibiotic or

PDI) had low effectiveness and, therefore, the effect was not enough to promote a significant bacterial inactivation even when combining the therapies.

5. Conclusion

The concentration of antibiotics and/or PDI that are not/very efficient in monotherapy tends to present antagonistic results. The most strongly synergistic conditions were identified for GEN, which did not show any antagonistic results.

Disclosures if required

The authors declare that there is no conflict of interest.

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Antimicrobial photodynamic response in *Rhyzopus oryzae*

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Mucormycosis is an extremely aggressive fungal disease that has a high mortality rate. Conventional treatment consists of high doses of antibiotics and antifungal agents associated with surgical resections, but even with this aggressive treatment, the mortality rate is still high. The antimicrobial photodynamic therapy (aPDT), also called photodynamic inactivation (PDI), it is an interesting alternative to antifungal therapy because it has non-specific biological site action and can also be an additional method of treatment in conjunction with surgery and antifungal therapy. In this work we studied the evaluation of the photodynamic antimicrobial response in different phases of fungus growth (conidium and hyphae in the white and black phases) and for different protocols of PDI with PDZ, varying the concentration of photosensitizer, the incubation time, the association with surfactant and the fluence of light at 660 nm. In this work, PDI showed great potential in the in vitro study of *R. oryzae*.

Keywords: Mucormycosis. *Rhizopus oryzae*. Photodynamic inactivation. Microbial control

1. Introduction and Background

During the COVID-19 pandemic, several secondary infections appeared in patients, one of them being mucormycosis, which is an extremely aggressive fungal disease with a high mortality rate. In October 2022, the WHO released the first list of fungi that pose a great risk to public health due to the invasive way these fungal diseases affect debilitated patients, one of the fungi cited is Rhizopus spp. which causes mucormycosis [1]. PDI has been presented as an alternative treatment to the conventional one in the treatment of mycoses and may be a potential coadjuvant alternative for microbial control of *Rhizopus spp.*

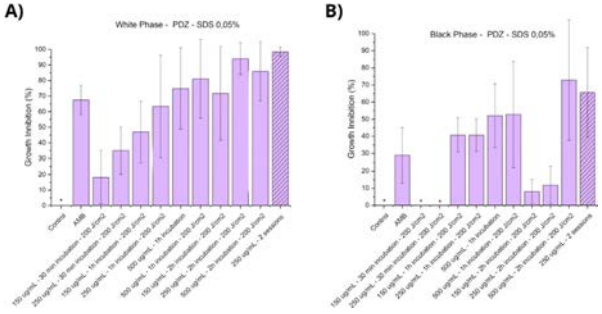
2. Aims

The purpose of this study is to evaluate the efficiency of photodynamic inactivation in the growth of *R. oryzae*.

3. Methods

Eight mm diameter discs were removed from 24-hours (light phase) and 48-hours (dark phase) cultured plates using a sterile syringe. Then, the disks were inserted into a well of a 24-well plate. For the PDI protocol PDZ was used, several protocols were tested varying incubation times from 20 minutes to 2 hours, PDZ concentrations, and association with the surfactant Sodium Dodecyl Sulfate (SDS). PDZ was tested in concentrations ranging from 150 μg/mL to 2.6 mg/mL. A light device at 660 nm and fluence between 100 and 200 J/cm^2 was used to perform the PDI irradiation. After treatment, the growth inhibition rate was calculated. The light and black phases samples were analyzed with Confocal Fluorescence Microscopy to obtain images of the fungus autofluorescence, bright field images, to observe cell structures, PDZ internalization and damage after aPDT. Samples were also analyzed by Transmission Electron Microscopy to study subcellular structures of *R. oryzae.* PDI experiments were performed on conidium suspension, using PDZ, varying the concentration from 25 to 150 μg/mL and fluence 100 J/cm^2.

4. Results



The combination of PDZ and 0.05% SDS showed a positive PDI response in *R. oryzae* light phase samples. A 94 % inhibition in the 1 session protocol and for the 2 sessions protocol, 98% growth inhibition was achieved. A 72 % inhibition was observed in the 1 session PDI protocol, while for the 2 sessions protocol 65 % growth inhibition was obtained. Using confocal microscopy, it was noted that for the white phase of growth of *R. oryzae*, the PDZ was distributed heterogeneously in some regions and homogeneously in others, in the hyphae, while in the black phase the PDZ is distributed completely heterogeneously. After the PDI it was observed that the hyphae showed a change in their structure, presenting an irregularity in the structure of their cell wall, showing the damage induced in the cell wall, it is also noted that there was a rupture of some hyphae, and extravasation and deposition of amorphous