TITLE: PHOTODYNAMIC TREATMENT IMPAIRS METABOLISM IN BIOFILMS OF PATHOGENIC FUNGI

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ABSTRACT:
Aspergillus flavus and *Aspergillus fumigatus* are the main etiological agents of aspergillosis and are also important producers of mycotoxins. These fungi are capable of developing as biofilms, which are complex and heterogeneous cellular communities made of conidia and hyphae firmly adhered to a solid surface and to each other. Due to its structure and properties, biofilms are also more resistant to antimicrobials, which often results in the failure of conventional antifungal therapy. The necessity of overcoming this issue and of controlling biofilm-forming microorganisms has led to the search for alternative therapeutic strategies, such as Antimicrobial Photodynamic Treatment (APDT). Previous studies have shown the efficacy of APDT against bacterial and yeast biofilms, but little is known about the effects of APDT on filamentous fungi biofilms. APDT is based on the use of a photosensitizer (PS) accumulating in the target microorganism which, upon illumination, reaches an excited state. The excited PS molecule then reacts with molecular oxygen via electron or energy transfer and produces reactive oxygen species, effectively killing the target pathogen. Our study evaluated the effects of APDT with phenothiazinium PS on the viability and metabolism of biofilms. Biofilms were developed on a polyethylene support (1 cm × 1 cm) for 36 h (*A. flavus*) or 48 h (*A. fumigatus*) in Khanna medium. APDT was carried out with the PS Methylene Blue (MB) (0.8 to 17 μg mL^{-1}) and New Methylene Blue (NMB) (1 to 25 μg mL^{-1}). Biofilms were irradiated using a LED array with maximum emission band at 631 nm and light fluences of 50 J/cm^2 and 100 J/cm^2. The growth and metabolic activity of the biofilms were measured, the latter using the Resazurin Reduction Test at 12, 24 and 36 h after APDT. Biofilms of both *A. flavus* and *A. fumigatus* continued to grow after APDT with either PS. However, we observed reductions in the metabolism of *A. flavus* biofilms after APDT with 17 μg mL^{-1} MB and with 25 μg mL^{-1} NMB. Also, metabolism of *A. fumigatus* biofilms after APDT with NMB was significantly lower when compared to biofilms treated with MB, at all concentrations evaluated. Even though biofilm growth could not be inhibited, our results show that APDT impaired biofilm metabolism, indicating that optimization efforts could eventually allow biofilm control by this strategy.

Keyword: Antimicrobial photodynamic inactivation, biofilms, A*spergillus flavus*, A*spergillus fumigatus*, methylene blue, new methylene blue.

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