A pilot study of antimicrobial photodynamic therapy of encapsulated Aspergillus fumigatus in a rabbit maxillary sinus model

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INTRODUCTION
Aspergillus fumigatus is a commonly isolated agent in invasive aspergillosis and chronic invasive fungal rhinosinusitis. These conditions, in particular, are often associated with immunosuppression. Standard management involves surgical debridement and long-term antifungal treatment (3–15 months, follow-up <5 yr), along with tight glycemic control and discontinuation of corticosteroids. Prompt diagnosis and initiation of appropriate therapy for relapse are essential to avoid a protracted or fatal outcome. This study was undertaken to demonstrate the potential for antimicrobial photodynamic disinfection (PDD) in the treatment of aspergillosis.

METHODS

Agar bead preparation (Fig. 1). A. fumigatus (ATCC 32820) conidia were harvested, added to warm TDP media with agar and dispersed in 95°C mineral oil, forming microbeads. After cooling, the solution was added to PBS, centrifuged and washed 3 times, and filtered through a 250 μm mesh filter prior to use.

PDD, planktonic model. 20 μL of 10^6 conidia/ml was added to a stock solution of photosensitizer containing 0.03% methylene blue chloride (PS) in USP water. 670 nm illumination was conducted for 60 s at an intensity of 150 mW/cm^2 while stirring. Samples were removed for PDA plate enumeration.

PDD, biofilm model (Fig. 2). 200 μL of 10^6 conidia/ml were added to each well of a 96-well plate and incubated while shaking at 125 rpm at 37°C for 48 hr. The resulting biofilm was washed 3X with PBS and 200 μL of PS added to each well for 4 min. Residual PS was removed and illumination conducted for 8 min at an intensity of 150 mW/cm^2. Swab samples were taken for PDA plate enumeration.

In vitro PDD (Fig. 3). Bead-encapsulated A. fumigatus was inoculated into the NZW rabbit maxillary sinus. No immunosuppression was required. After 48 hrs, PS was applied to the affected sinus followed by 670 nm illumination at 150 mW/cm^2 for 8 min via a custom diffuser.

CONCLUSIONS
Because fungi are present throughout the environment, human exposure is inevitable and normal respiration will routinely deposit fungal elements within the nose, paranasal sinuses, and in the remainder of the airway. In cases of immunosuppression or otherwise poor health, invasive aspergillosis may result in lethal outcomes. Current approaches to treatment using azoles may result in severe hepatotoxicity and steroid treatment can induce diabetes and other negative sequelae. This pilot study demonstrated that topical in-vivo antimicrobial photodynamic therapy was capable of eradicating >3 log_10 (>99.9%) of A. fumigatus inoculated into the NZW rabbit maxillary sinus in a recognized agar bead model of aspergillosis. This represents a >4,000-fold reduction in the number of viable conidia per unit area. These in vivo results closely matched in vitro experimental data in planktonic and biofilm cultures. This pilot study will be replicated in a larger sample size and with polysorbial cultures of important human fungal pathogens including A. nigra, A. flavus and M. indicus.

REFERENCES

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