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Amphotericin B releasing nanoparticle topical treatment of *Candida spp.* in the setting of a burn wound

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Abstract

Candida spp. infection in the context of burn wounds leads to invasive disease with a 14–70% mortality rate. Unfortunately, current administrations of AmB, an important therapeutic demonstrating minimal resistance, are only available via potentially cytotoxic IV infusions. In order to circumvent these sequelae, we investigated the efficacy of nanoparticle encapsulated AmB (AmB-np) as a topical therapeutic against *Candida spp.* (drug release equilibrated solubilized AmB [AmB-sol] included as control). Clinical strains demonstrated equal or enhanced killing efficacy with 72.4–91.1% growth reduction by 4 hours. AmB-nps resulted in statistically significant reduction of fungal biofilm metabolic activity ranging from 80% to 95% viability reduction ($P<0.001$). Using a murine full-thickness burn model, AmB-np exhibited a quicker efficiency in fungal clearance versus AmB-sol by day three, although wound healing rates were similar. These data support the concept that AmB-np can function as a topical antifungal in the setting of a burn wound.

From the Clinical Editor: The control of fungal infections with *Candida* species remains a challenge in the context of burn wounds. A nanoencapsulated topical amphotericin-B compound was studied in a murine model of full thickness burn injury, showing remarkable efficacy in controlling *Candida* infection. This may become a viable alternative to the potentially toxic intravenous formulations.

Key words: Nanoparticles; Topical antifungal; Burn wounds; *Candida spp.*; Amphotericin B

Despite significant advances in the treatment of severe thermal injury, infection and sepsis persist as frequent causes of morbidity and mortality for burn victims due to extensive compromise of the skin and contiguous tissue that serve as a protective barrier against microbial invasion.[1–3] Furthermore, thermal injury impairs a robust immune response during an infectious process due to the attenuation of dermal defense mechanisms such as keratinocyte-released defensins and acidic secretions derived from sebaceous glands (i.e., fatty and lactic acids).[4] Burn-induced immunosuppression facilitates localized colonization and systemic entry of microbes from both endogenous and exogenous origins.[2,5,6]

In the setting of a burn wound infection, *Candida spp.* are etiologic in approximately 8% of cases, with non-albicans *Candida* (NAC) species demonstrating overall higher rates of mortality in comparison to *Candida albicans,* (54% and 37%, respectively).[5] *Candida* spp. are the fourth most commonly isolated pathogens from bloodstream infections in US hospitals.[7,8] Although *C. albicans* remains the most commonly isolated species, NAC species are increasingly important, as they have been implicated in 35–65% of all candidemias within the general patient population.[9]

The optimization and standardization of treatment for invasive *Candida*isis as a consequence of thermal injury is imperative as mortality varies extensively (14–70%) within dedicated burn intensive care centers.[8] Rates of fungemia following traumatic burns have increased or remained stagnant since the inception of topical burn wound chemotherapeutic agents such as silver sulfadiazine dressing.[1,2,10,11] Amphotericin B (AmB), a potent antimycotic polyene macrolide, is an

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intravenously administered first-line treatment for burn related fungemia attributed to *Candida spp*. as well as many moulds.[8] AmB-sol (a formulation of amphotericin B conjugated to sodium deoxycholate) has long been regarded as the gold standard for severe systemic mycoses (especially for administration prior to fungal identification and susceptibility results) due to its remarkably low level of resistance amongst fungal species and proposed fungicidal mechanisms that account for broad-spectrum coverage. However, the associated dose-dependent nephrotoxic agent has mitigated its usage – as many as 80% of recipients present with adverse clinical reactions within two weeks of administration.[12] Therefore, when feasible, clinicians use less toxic, but costly lipid-conjugated formulations of AmB.[8]

Topical administrations of antimicrobials are advantageous for effective treatment of locally invasive disease due to their inherent ability to circumvent systemic cytotoxicity and ease of rapid delivery at the site of infection.[13] Unfortunately, potent antifungal therapies such as AmB, are only available in parenterally administered formulations due to their large, hydrophobic chemical structure that hinders adequate cutaneous penetration. Thus, we incorporated AmB into an inexpensive, silane-based hydrogel nanoparticle platform that putatively results in controlled drug release once exposed to an aqueous milieu.[14–16] Several clinical *Candida spp.* strains were subjected to amphotericin B nanoparticle (AmB-np) treatment *in vitro* in an effort to validate AmB-np's antifungal efficacy. In view of our hypothesis that this unique nanoparticle system can effectively transport encapsulated molecules across diverse and complex biological barriers, we tested the efficacy of the AmB-np as a vector for cutaneous AmB delivery in the setting of a murine burn wound infection with *C. albicans*.

Methods

Candida spp. strains

C. albicans SC5314 was obtained from M. Ghannoum (Cleveland, OH). *C. albicans*, *C. glabrata*, and C. *parapsilosis* clinical strains were procured from Montefiore Medical Center, Bronx, NY, and All India Institute of Medical Science, New Delhi, India, courtesy of P. Gialanella (Bronx, NY) and B. Fries, respectively (Bronx, NY). Quality control ATCC strains 225019 (*C. parapsilosis*) and 6258 (*C. krusei*) were acquired from the American Type Culture Collection (Manassas, VA). For all experiments, the isolates were grown in yeast extract, peptone, and dextrose (YPD) broth (Difco Laboratories) containing 10 g/L yeast extract, 10 g/L peptone, and 20 g/L glucose for 24 h at 30°C with shaking at 150 rpm (to early stationary phase).

Amphotericin B Nanoparticle synthesis

For the incorporation of AmB into a nanoparticle delivery system, we modified the previously described technique utilized to successfully nano-encapsulate nitric oxide.[14,15] For AmB-np, 500 μL of polyethylene glycol, 500 μL of chitosan (5mg/mL, pH 5) and 10 mL of AmB (250 μg/mL) (Sigma, St. Louis, MO) were added to sonicated solution prior to lyophilization. The resulting hydrogel/glassy composite nanoparticle powder sequesters our drug of interest until being exposed to an aqueous milieu for uniform release.

Particle sizing of AmB-np

Each particle sizing measurement using dynamic light scattering required a 10 μL sample of 1 mg/mL of AmB-np suspended in PBS; all readings were executed in triplicate and compiled with an acquisition length of 5 seconds and a total of 40 acquisition attempts (DynaPro™ Nanostar, Wyatt Technology, CA).

AmB-np release kinetics

Nanoparticles (1 mg/ml) were suspended in phosphate buffered solution (PBS, pH 7.4) and incubated at room temperature and shielded from light on a rotator for varying lengths of time. Samples were taken at 0, 10, 30, 60, 120, 240, and 1440 minutes post-suspension and analyzed by UV–vis spectroscopy. The concentration of AmB in the AmB-np suspension was determined by analyzing a solution with a standard concentration (25 μg/ml) of AmB using the following conversion equation:

$$\text{Conc. from NP release} = \frac{\text{Abs}_{\text{NP@409nm}}}{\text{Abs}_{\text{StdConc.@409nm}}} \times \text{Conc. from Std}$$

Total release of AmB from the nanoparticle solution was further substantiated by minimal inhibitory concentration assays (MICs) utilizing ATCC *Candida spp.* standards with recorded antifungal susceptibilities. AmB-sol and AmB-np were tested against quality control ATCC strains 225019 and 6258 to confirm whether nanoparticle release corresponds to release kinetics data extrapolated from UV–vis spectroscopy. MICs were conducted following Clinical Laboratory Standards Institute guidelines for in vitro *Candida spp*. susceptibility testing that included AmB-sol and blank nanoparticle (blank-nps) controls.[17]

Determining cytotoxicity exerted by AmB-np

Cytotoxicity/cytolysis was measured via a colorimetric lactate dehydrogenase assay (LDH) (Cytotoxicity Detection Kit^{PLUS}, Roche, Mannheim, Germany). J774.16 peritoneal murine macrophages (10^6) were plated and cultured overnight at 37 °C, 5% CO_2. Macrophages were then subjected to varying concentrations of AmB-np suspended in feeding media for 2 h. Cells were then spun down for collection of supernatant to measure LDH release.

Metabolic viability and growth assays using AmB-np against Candida spp

Using the semiquantitative XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) metabolic assay, AmB-np's capacity to inhibit *Ca* SC5314 viability was assessed. In a 96 well-plate, 10^6 *C. albicans* cells were inoculated in 100 μL of YPD and 100 μL of 1 mg/mL of AmB-np with different plates representing a time point (2, 4, 6, 8, 24 h). Equilibrated AmB-sol treatment and untreated wells served as controls. XTT assay was conducted as previously described.[18,19]