Abstract
Rhizopus delemar is an opportunistic fungal pathogen and the primary causative agent of mucormycosis, an invasive infection with mortality rates often exceeding 50%. Hallmarks of mucormycosis include angioinvasion and the production of a ricin-like toxin (mucorcin). Treatment usually involves repeated injections of fungicidal amphotericin B (AmB). However, AmB’s extended use is restricted by its severe toxicity concerns. Our research group has developed a novel technology in which C-type lectin receptors, which recognize fungal cell oligosaccharides, are incorporated onto the outer surface of an antifungal-loaded liposome (DectiSomes). These immune receptors guide the liposomes to fungal cells, concentrating them away from human cells and thus minimizing potential toxicity effects. I am currently evaluating the efficacy of these DectiSomes against R. delemar. DectiSomes bind to R. delemar hyphae at least orders of magnitude more effectively than uncoated antifungal-loaded liposomes. Preliminary data indicate that DectiSomes also effectively inhibit fungal metabolic activity in vitro and reduce lung fungal burden in a neutropenic mouse model of pulmonary mucormycosis. We continue to explore the potential of this exciting novel therapeutic for treating mucormycosis.

Fig. 1. DectiSomes are C-type lectin-coated, antifungal-loaded liposomes that specifically target fungal cells. A. DectiSOME model. In this example, an Amphotericin-B loaded liposome is coated with the carbohydrate recognition domains (CRDs) of Dectin-1 monomers. These monomers form dimers that bind to the fungal target of interest. Rhodamine is included for microscopic detection of liposomes. B. DectiSOME concept. Un-targeted antifungal liposomes enable the diffusion of the antifungal to both fungal cells and host cells (left), whereas DectiSomes promote a concentrated and localized diffusion of the antifungal in proximity to the fungal target of interest (right).

Fig. 2. Dectin-1 coated liposomes bind to fixed R. delemar hyphae. AmB-loaded liposomes that were coated with Dectin-1 CRD monomers (DECI-AmB-LLs (C)) bound orders of magnitude more efficiently to fixed R. delemar hyphae compared to uncoated AmB-loaded liposomes (AmB-LLs) (A) or BSA-coated, AmB-loaded liposomes (BSA-AmB-LLs) (B). Images were taken at 5X magnification. D. The area of red fluorescence for each set of images (N = 10) was quantified and plotted (log(10)).

Fig. 3. Lamrinarin inhibits DECI-AmB-LLs binding to R. delemar. DECI-AmB-LLs (A) were co-incubated with either yeast mannans (B) or laminarin (C) prior to addition to R. delemar culture to verify the specificity of Dectin-1 binding activity to fungal β-glucans. Images were taken at 5X magnification. D. The area of red fluorescence for each set of images (N = 7) was quantified and plotted.

Fig. 4. DECI-AmB-LLs bind to live R. delemar hyphae. DECI-AmB-LLs (C) bound orders of magnitude more efficiently to live R. delemar hyphae compared to AmB-LLs (A) or BSA-AmB-LLs (B). Images were taken at 5X magnification. D. The area of red fluorescence for each set of images (N = 7) was quantified and plotted (log(10)).

Fig. 5. DECI-AmB-LLs inhibit R. delemar metabolic activity at low drug concentrations. DECI-AmB-LLs were more effective at inhibiting the metabolic activity of R. delemar in vitro compared to AmB-LLs and BSA-AmB-LLs. This effect was observed at relatively low drug concentrations of AmB.

Fig. 6. DECI-AmB-LLs reduce the lung fungal burden in a pulmonary mucormycosis mouse model. Quantitative PCR analysis of R. delemar 18S RNA from mice lungs indicate that DECI-AmB-LLs significantly reduce the lung fungal burden in a pulmonary mucormycosis mouse model compared to AmB-LLs at a low drug concentration.

Conclusions
DectiSomes are immune receptor-coated, antifungal-loaded liposomes that specifically target fungal cells. Dectin-1-coated, AmB-loaded liposomes (DECI-AmB-LLs) specifically bind to β-glucans found on Rhizopus delemar hyphae. DECI-AmB-LLs also inhibit the metabolic activity of R. delemar and reduce the lung fungal burden in infected mice at moderately low drug concentrations.

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Ethical considerations
All protocols met guidelines for the ethical treatment of non-human animals outlined by the U.S. Federal government and were approved by UGA’s Institutional Animal Care and Use Committee as described in our animal use protocols. AUPs A2019 03-01-A1 and A2018 12-009-Y2-A2.

Targeted delivery of antifungal liposomes to *Rhizopus delemar*

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