Efficacy of Aerosol AmBisome® with and without Intravenous AmBisome Treatment Against Different Strains of *Aspergillus fumigatus*

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ABSTRACT

Aspergillus fumigatus **causes nearly 90% of invasive pulmonary aspergillosis (IPA) infections with 50% mortality even after intravenous (IV) AmBisome® (AmBi) treatment. Our lab previously demonstrated that by aerosolizing (aero) AmBi and using it in combination with IV AmBi, efficacy against murine IPA caused by *A. fumigatus* strain 13073 was better than using IV AmBi alone. Since IPA patients are infected by different strains of *A. fumigatus*, we used this combination approach to see if it would be effective in treating IPA caused by strains V080 and V029.**

Swiss-Webster female mice were immunocompromised with triamcinolone acetonide (28mg/kg d-3, -1, +1), challenged d0 with 3.2x10ex7 *A. fumigatus* V029 or 2.2x10ex7 *A. fumigatus* V080 and then divided into 4 groups/strain (n=7/group) receiving either aero AmBi (1.33 mg/mL for 20 minutes for 5 days), aero + IV AmBi (7.5 mg/kg for 3 days), IV AmBi (7.5 mg/kg for 3 days), or IV buffer. Aero AmBi was initiated 4h post-challenge and IV AmBi at 12h post-challenge. Mice were monitored for morbidity to d+21.

Aero AmBi monotherapy yielded 71% survival with V029 and 100% survival with V080. Combination therapy, however, resulted in even greater efficacy, giving 100% survival with V029 and 86% survival with V080. Survival was only 43% with IV AmBi monotherapy and 14% for buffer treated mice. Disease signs and weight loss paralleled survival. In conclusion, the combination of aero and IV AmBi is an effective approach for treating murine IPA caused by different *A. fumigatus* strains, indicating that this approach has the potential to increase AmBi outcomes in the clinical setting.

INTRODUCTION

Aspergillus spp. **is a medically and industrially important fungal genus that exists in many environments ranging from the soil, air, surfaces, food, water, and organic matter (Paulussen *et al.*, 2016). Within the *Aspergillus* genus there are hundreds of species, though only a few are responsible for having harmful effects on human and animal health. *A. fumigatus* is one of these and is responsible for nearly 90% of all invasive pulmonary aspergillosis (IPA) infections, followed in frequency by *A. flavus* and *A. niger* (Lass-Flörl *et al.*, 2005; Balajee *et al.*, 2009a,b).**

IPA is most prevalent in immunocompromised individuals, with a 50-90% mortality rate without treatment and a 50% mortality rate even with present treatment, indicating the need for improved therapy (Dagenais *et al.*, 2009; Shaunak *et al.*, 2015). Even more concerning, the rates of IPA have increased dramatically in recent years with the expanding use of immunosuppressive agents used for transplantation and cancer chemotherapy (Paulussen *et al.*, 2016). Present treatment options for IPA include intravenous amphotericin B based formulations. Among these is intravenous AmBisome® (AmBi), a potent, antifungal, liposomal formulation of amphotericin B, used as first-line therapy for IPA given its significantly reduced toxicity compared to the other amphotericin B formulations (Patterson *et al.*, 2016).

To further reduce any possible systemic toxicity from IV delivered AmBi, other routes of administration have been investigated. Preclinical studies using AmBi as an aerosol, rather than as an intravenous treatment, have shown its potential for treating IPA in immunosuppressed mice (Dave *et al.*, 2019). We recently completed a series of novel studies investigating the use of AmBi as an aerosol compared to intravenous delivery. Our laboratory has reported that aerosol delivery of AmBi achieved antifungal lung drug concentrations effective against IPA in immunosuppressed mice when the causative pathogen was *A. fumigatus* (ATCC strain #13073) (Dave *et al.*, 2019). This study also showed that AmBi given intravenously produced higher concentrations of drug in the kidneys, liver, spleen, and lungs versus aerosol delivery, but aerosol AmBi increased survival and reduced lung fungal burden significantly better than when AmBi was given by the intravenous route.

Although aerosol AmBi is highly effective in this mouse model of IPA using one particular strain of *A. fumigatus*, multiple strains of *A. fumigatus* as well as other species of *Aspergillus* need to be tested in this same murine model. Using multiple strains that cause aspergillosis will help determine if aerosol AmBi can be used more widely since the causative strain of fungus varies greatly based on geographical location. Data from published literature has reported significant differences in virulence of different strains of *A. fumigatus* and differing responses to anti-fungal drugs by other *Aspergillus spp.* (Latgé, 1999). The focus of the present studies was to determine if aerosol AmBi delivery alone or in combination with IV AmBi can be used to treat IPA caused by different strains of *A. fumigatus.*

METHODS

In this study, 8-9 week old Swiss-Webster mice were injected intraperitoneally with the steroidal immunosuppressant, triamcinolone acetonide and subsequently inoculated intranasally with 30 μL of 3.2x10^7 *A. fumigatus* V029 or 2.2x10^7 *A. fumigatus* V080. Mice were separated into 5 groups (n=7/group) and received AmBi as follows:

- **Aerosol AmBi for 5 daily doses, 20 minutes each, beginning 4h post-challenge**
- **Aerosol AmBi for 5 daily doses, 20 minutes each, beginning 8h post-challenge**
- **Aerosol AmBi for 5 daily doses, 20 minutes each, beginning 8h post-challenge and IV given at 12h, 36h, 60h post-challenge**
- **IV AmBi for 3 daily doses, given at 12h, 36h, and 60h post-challenge**
- **IV Buffer for 5 daily doses, beginning 4h post-challenge**

The above mice from each treatment group were monitored for survival, weight loss and disease signs for 21 days after the fungal challenge.

RESULTS

Figure 1a. Survival of Mice Infected with *A. fumigatus* V029: Combo therapy with aero + IV AmBi® results in Best Survival

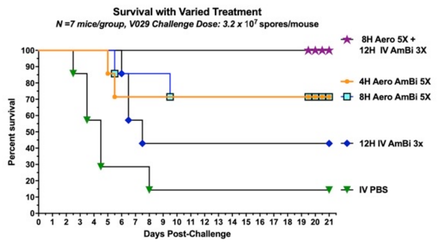


Figure 1b. Survival of Mice Infected with *A. fumigatus* V080: Daily 20min. Aero Ambi® Beginning 4h Post-Challenge Results in Best Survival

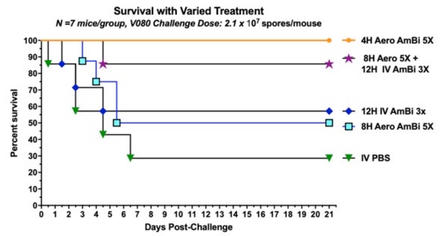


Figure 2a. Weight Loss of Mice Infected with *A. fumigatus* V029 with Varying Treatments for Pulmonary Aspergillosis: Only Aero + IV AmBi Therapy Resulted in Full Weight Recovery

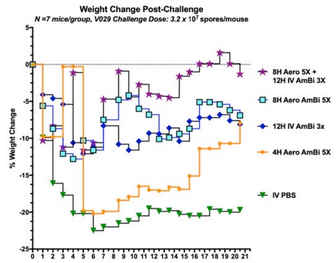


Figure 2b. Weight Loss of Mice Infected with *A. fumigatus* V080 with Varying Treatments for Pulmonary Aspergillosis: Daily 20min. Aero AmBi and Aero + IV AmBi Therapy Resulted in Full Weight Recovery

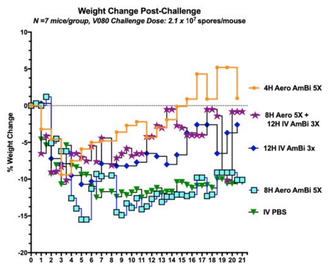


Figure 3a. Disease Scores of Mice Infected with *A. fumigatus* V029 with Varying Treatments for Pulmonary Aspergillosis: Aero + IV AmBi Therapy Results in the Lowest Disease Scores

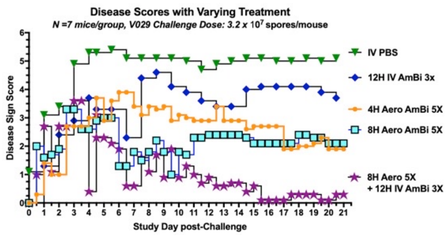


Figure 3b. Disease Scores of Mice Infected with *A. fumigatus* V080 with Varying Treatments for Pulmonary Aspergillosis: Daily 20min. Aero AmBi Beginning 4h Post-Challenge Results in the Lowest Disease Scores

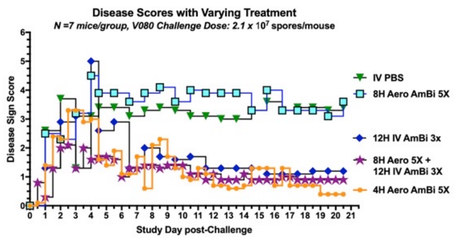


Figure 4. Aerosol Apparatus

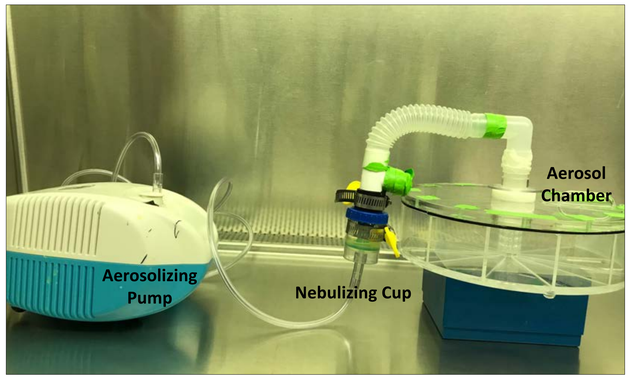
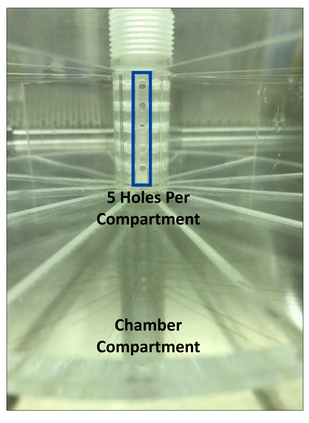


Figure 5. Inner Chamber View of Aerosol Point of Entry



CONCLUSIONS

In mice challenged with *A. fumigatus* strain V029:

- **Aero AmBi in combination with IV AmBi had the highest survival and lowest disease scores and weight loss.**
- **Mice given aero AmBi monotherapy 4H post-challenge had similar efficacy in survival and low disease scores and weight loss**

In mice challenged with *A. fumigatus* strain V080:

- **Aero AmBi monotherapy 4H post-challenge had the highest survival and lowest disease scores and weight loss.**
- **Mice given aero AmBi in combination with IV AmBi had similar efficacy in survival and low disease scores and weight loss.**

Future Studies

- **Determine the organ drug concentrations and organ fungal burden of mice treated with the same treatment groups as used in this present survival student.**
- **Use Immunohistochemistry to determine localization of drug in lungs following Aero AmBi.**

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