Aerosolization of Liposomal Amphotericin B (AmBisome®) Maintains Antifungal Drug Concentrations in Lungs for up to Five Days Post-Treatment

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ABSTRACT

Background: In immunocompromised patients, Aspergillus causes severe pneumonia with 50-90% mortality in untreated patients and 50% mortality even with treatment. The high patient mortality indicates that treatment for this disease needs to be improved. Although AmBisome® (AmBi) a potent antifungal drug, is used intravenously (IV) to treat this infection, aerosol AmBi treatment has the potential to deliver the drug to the lungs at greater concentrations and be used alone or in combination with IV AmBi for treatment. We developed an aerosol delivery system for AmBi and evaluated its pharmacokinetics in mice to determine baseline drug levels for future treatment of pulmonary aspergillosis.

Methods: AmBi (1.33 mg/mL) was nebulized with a Schuco S5000 nebulizer and delivered to mice in a compartmentalized, isolation chamber. Swiss-Webster female mice received one, two or three daily, twenty-minute aerosol treatments (12 mice/treatment group). Mice (n=4/treatment group/time point) were sacrificed at 24,72 or 120 hours after their last aerosol treatment. The lungs, livers, kidneys and spleens were collected, homogenized, and amphotericin B extracted from the tissues via methanol, and analyzed for drug concentration via bioassay using AmBi standards and *Candida albicans* as the indicator organism.

Results: The aerosol system was successful in delivering at least the MIC level (2-4 μg/g) for Aspergillus spp. in the lungs of all treatment groups at all time points. The mean lung drug concentration after 24h was 17.7, 5.34, and 3.64 μg/g for three, two or one treatments, respectively, and the concentrations decreased over time to 11.13, 3.04, and 3.69 μg/g, respectively, after 120h. None of the kidneys and livers from any treatment group contained detectable levels of amphotericin B. Only the spleen had detectable drug levels, but only at 120h after one, two or three treatments (1.53, 1.64 and 3.33 μg/g, respectively).

Conclusions: Aerosol delivery after three treatments with AmBi achieved lung drug concentrations in mice well above the MIC for many Aspergillus spp. (17.7 μg/g) indicating that this route of administration could be used to treat pulmonary aspergillosis. The low concentrations of amphotericin B in the kidneys and livers following aerosol delivery is advantageous since AmBi can be associated with some limited nephrotoxicity and hepatotoxicity.

INTRODUCTION

In Immunocompromised patients, infection by Aspergillus species has life threatening effects, it causes severe pneumonia and results in patient mortality in at least 50% of treated patients and 50-90% of untreated patients (Shaunak *et al*., 2015). The immunocompromised population susceptible to infection by Aspergillus species is comprised of a wide array of patients such as those receiving bone marrow and organ transplants as well as patients receiving treatment for certain cancers, severe burns, and AIDS. Fungal infections such as aspergillosis can kill patients who have otherwise been successfully treated for the condition that resulted in their initial hospitalization. The extensive complications caused by invasive aspergillosis and the high mortality associated with this infection makes the study of its treatment very important.

AmBisome, the lipid formulation of Amphotericin B, is one of the first-line therapies for treating invasive aspergillosis (Patterson *et al*., 2008). AmBisome is significantly less toxic and better tolerated than other forms of amphotericin B therapy. Several studies have shown the prophylactic use of aerosol AmBisome therapy in preventing invasive pulmonary aspergillosis and in clinical trials, patients experienced no serious adverse events due to the inhalation of AmBisome (Rijinders *et al*., 2015). It is possible that aerosol treatment could be more effective for treating pulmonary fungal infections as it allows for the drug to concentrate in the lungs which is the place where most infections occur (Schiller, 2015).

In order to study the potential for aerosol therapy in treating pulmonary aspergillosis in people, an animal model has to be used as a critical step in evaluating different dosing regimens and different doses of the drug to maximize the feasibility of this approach. To investigate this we standardized an aerosol delivery system for AmBisome (liposomal amphotericin B) in mice, and compared how much drug was delivered to the lungs, liver, kidneys and spleen following different numbers of treatment.

METHODS

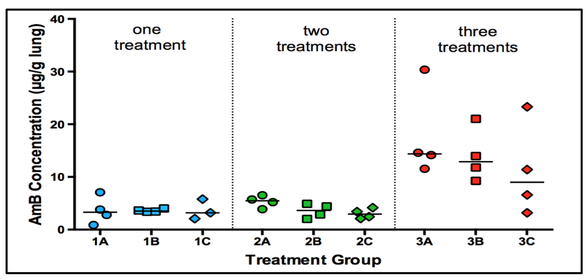
1) Non-sedated, Swiss Webster female mice, ages 5-6 weeks old, were divided into 12 mice/group, with one group receiving one treatment, another group receiving two treatments and another group receiving three treatments of Aerosolized AmBisome (1.33 mg/mL in reservoir) for 20-minutes intervals spaced 24h apart.
2) Mice were weighed once daily, prior to daily treatment . It was ensured that mice were consistently placed in the same isolation chamber compartments each day for treatment.
3) After receiving their last aerosol AmBi treatment, mice were euthanized at 24h(n=4), 72h(n=4) and 120h(n=4) by carbon dioxide asphyxiation and the lungs, spleens, livers and kidneys were collected.
4) Tissue samples were weighed using tared tubes, and stored at -80 °C.
5) Tissues were then homogenized in 1 mL of PBS with 0.05% Chloramphenicol, and amphotericin B was extracted from the homogenate using methanol, heating at 60 degrees Centigrade for 10 minutes, centrifuging and collecting the supernatant.
6) The supernatant was analyzed via a bioassay, using *C. albicans* as the indicator organism and expressed as μg/g tissue.

RESULTS

Lungs 1,2 or 3 Treatments			Three Aerosol Treatments	Two Aerosol Treatments	One Aerosol Treatment
Group	Time Sacrificed	Mouse	Amp B Concentration (μg/g)	Amp B Concentration (μg/g)	Amp B Concentration (μg/g)
A	24h	1	14.15	6.52	7.08
	24h	2	11.56	3.89	3.82
	24h	3	14.60	5.22	0.88
	24h	4	30.36	5.72	2.78
Avg.			**17.67**	**5.34**	**3.64**
B	72h	5	21.02	2.88	3.4
	72h	6	11.80	2.03	3.64
	72h	7	13.97	4.91	3.43
	72h	8	9.26	4.41	4.02
Avg.			**14.01**	**3.56**	**3.63**
C	120h	9	11.39	3.42	5.79
	120h	10	23.324	2.11	Below Detection Limit
	120h	11	3.20	2.45	3.2
	120h	12	6.61	4.18	2.07
Avg.			**11.13**	**3.04**	**2.77**

Table 1. *Amphotericin B Concentration in Lungs of Immunocompetent mice at 24h, 72h, or 120h following one, two or three aerosol treatments.* With all treatments AmBi concentrations reached well above the MIC for most fungi (2 - 4 μg/ml), and slowly decreased after 72h.

Figure 1. *Concentration of Amphotericin B in Lungs of Immunocompetent Mice at 24h,72h, or 120h following one, two or three aerosol treatments:* Three treatments delivered the highest amount of AmBi to the lungs; one or two treatments had similar concentrations of drug with clearance after 24h. Unlike the liver and the kidney, only the lungs and spleens had detectable levels of AmBi.



Spleens 1,2, or 3 Treatments			Three Aerosol Treatments	Two Aerosol Treatments	One Aerosol Treatment
Group	Time Sacrificed	Mouse	Drug Concentration (μg/g) via Bioassay	Drug Concentration (μg/g) via Bioassay	Drug Concentration (μg/g) via Bioassay
A	24h	1	0	0	0
	24h	2	0	0	0
	24h	3	0	0	0
	24h	4	0	0	0
B	72h	5	0	0	0
	72h	6	0	0	0
	72h	7	0	0	0
	72h	8	0	0	0
C	120h	9	2.67	2.04	1.50
	120h	10	3.13	1.41	1.50
	120h	11	3.59	1.60	1.60
	120h	12	3.94	1.51	1.50
Avg.			**3.33**	**1.64**	**1.53**

Table 2. *Amphotericin B Concentration in Spleens of Immunocompetent mice at 24h, 72h, or 120h following one, two or three aerosol treatments.* For all treatment groups, AmBi was found in the spleen at only the 120h time point, suggesting that splenic uptake occured between the 72h and 120h time points.

Figure 2. *Concentration of Amphotericin B in Spleens of Immunocompetent Mice at 24h,72h, or 120h following one, two or three aerosol treatments.* For all treatment groups, AmBi was only detected at the 120h time-point in the spleens.

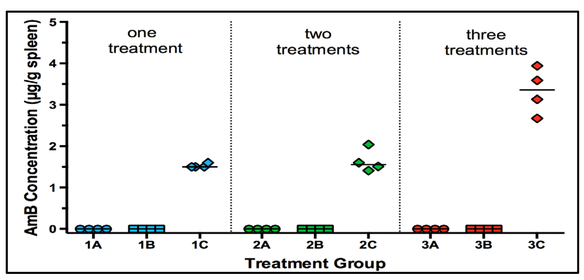


Figure 3. *Aerosol Apparatus*
Cal Poly Pomona sealed Aerosol Apparatus with Aerosol Chamber, Liquid Reservoir, and Schuco S5000 aerosolizing pump.

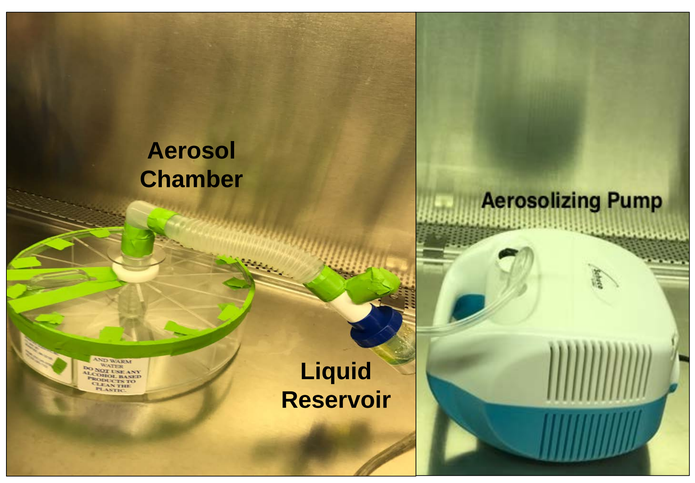


Figure 4. *View of Inside Chamber Compartment Showing Aerosol Point of Drug Entry into Compartment*

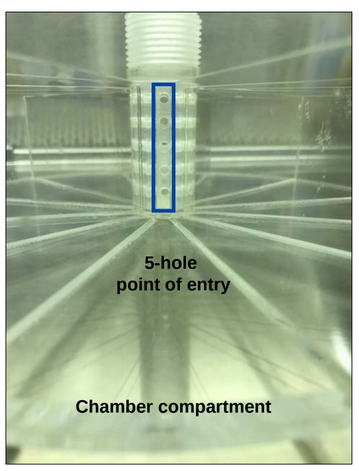


Figure 5. Live Mice in Each of 12 Compartments of Aerosol Chamber

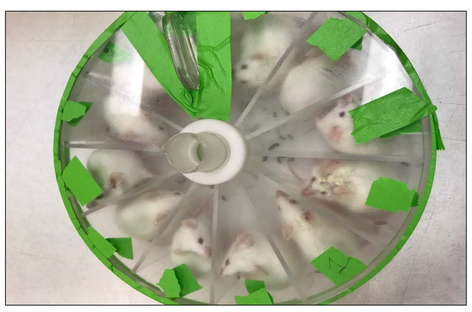
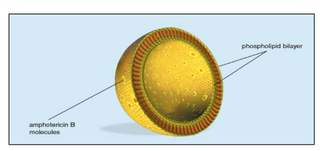


Figure 6. AmBisome Liposome



CONCLUSIONS

- The aerosol delivery method achieved amphotericin B drug concentrations at least 4x the MIC (2 - 4 μg/g) for Aspergillus species (17.67 μg/g with three treatments).
- With three treatments, amphotericin B was retained in the lungs for 24h, 72h, and 120h at concentrations of 17.67, 14.01, and 11.13 μg/g of tissue. The amphotericin B remained in the lungs well above the MIC for Aspergillus for at least 5 days.
- AmBisome was found in the spleens for all treatment groups only at 120h, suggesting delayed splenic uptake following aerosol administration.
- The aerosol apparatus delivered AmBisome at low concentrations to liver and kidney, which is advantageous since intravenous AmBi exhibits limited hepatotoxicity and nephrotoxicity.

Future Studies

- Determine the concentration of aerosolized AmBisome in immunosuppressed mice administered once every 24h, for three consecutive twenty-minute treatments.
- Investigate the efficacy, pharmacodynamics, and pharmacokinetics of aerosolized AmBisome alone or in combination with follow-up intravenous AmBisome for the treatment of pulmonary aspergillosis in Swiss-Webster mice.

REFERENCES

- Shaunak S, Armstrong, J.D., Teo I, Shirkhani K. 2015. Nebulised amphotericin B-polymetacrylic acid nanoparticle prophylaxis prevents invasive aspergillosis. Nanomedicine. 11(5):1217-1226.
- Patterson TF, *et al*. 2008. Treatment of Aspergillosis: Clinical Practice Guidelines of the Infectious Diseases Society of America. Clin. Infect. Dis. 46(3): 327-60.
- Rijinders B.J., *et al*. 2015. Aerosolized Liposomal Amphotericin B to Prevent Aspergillosis in Acute myeloid Leukaemia: Efficacy and cost effectiveness in real-life. Int. J. Antimicrob Agents. 46(1):82-7.
- Schiller D.S., Le, J. 2010. Aerosolized Delivery of Antifungal Agents. Current Fungal Infection Reports. 4:96-102.
- Smith, P., Olson, J., Constable, D., Schwartz, J.,Proffitt, R., Adler-Moore, J. Effects of Dosing Regimen on Accumulation, Retention and Prophylactic Efficacy of Liposomal Amphotericin B. Journal of Antimicrobial Chemotherapy. Vol. 59 (5): 941-951. 2007.

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