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APX001 Protects Immunosuppressed Mice from *Rhizopus delemar* Infection

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ABSTRACT

Objectives: Mucormycosis is a life-threatening infection with high mortality that occurs predominantly in immunocompromised patients. APX001A is an antifungal agent that targets Gwt1, an early step in the conserved glycosylphosphotidyl inositol (GPI) post-translational modification pathway of surface proteins in eukaryotic cells. Inhibition of inositol acylation by APX001A results in pleiotropic effects such as inhibition of maturation of GPI-anchored proteins necessary for growth and virulence and results in lethality. APX001A has *in vitro* activity against Mucorales. Here we assessed the *in vivo* activity of APX001, the prodrug of APX001A currently in clinical development, against *Rhizopus delemar* (MIC = 0.25 µg/mL).

Methods: ICR mice were immunosuppressed with cyclophosphamide (200 mg/kg) and cortisone acetate (500 mg/kg) on days -2, +3, and +8 relative to intratracheal infection with 2.5 x 10^5 cells of *R. delemar* 99-880. For survival studies, treatment with APX001 (prodrug) at 52, 104, or 156 mg/kg (twice daily, po), was compared to liposomal amphotericin B (LAmB) at 15 mg/kg (once daily, iv). Treatment started on day +1 through day +8 for APX001 and through day +4 for LAmB. Placebo mice received vehicle control. For fungal burden studies, dosing started 8 h post infection through day +3. Mice were sacrificed on day +4. Survival time, and tissue fungal burden (by qPCR) served as efficacy endpoints.

Results: APX001 treatment at either 52 or 104 mg/kg prolonged survival of mice vs. placebo (n=20 per arm) (21-day survival of 0% for placebo, 30% for 52 mg/kg, 45% for 104 mg/kg, P<0.05 by Log Rank test). APX001 at 104 mg/kg was as good as LAmB treatment (21-day survival of LAmB-treated mice [n=20] =50%). APX001 at 156 mg/kg did not enhance survival vs. placebo. Further, APX001 at 104 mg/kg and LAmB reduced pulmonary and brain fungal burden by ~1 log and 1.5 log vs. placebo, respectively (P<0.05, by Wilcoxon Rank Sum). The 52 and the 156 mg/kg APX001 doses also reduced tissue fungal burden vs. placebo mice (0.5-1.0 log).

Conclusion: APX001 protected immunosuppressed mice from *R. delemar* infection with efficacy similar to that of LAmB. Higher doses of APX001 were not protective despite lowering fungal burden. Continued investigation of APX001 as a novel antifungal agent against mucormycosis is warranted.

INTRODUCTION

- Mucormycosis (zygomycosis) is a rare but life-threatening fungal infection which are mainly caused by *Rhizopus* species (1).
- It occurs mostly in immunocompromised hosts such as neutropenic patients (1).
- APX001A is first-in-class antifungal that targets Gwt1, an enzyme required in the early steps of GPI post-translational modification of surface proteins in eukaryotic cells.
- Here we assessed the *in vitro* and *in vivo* activity of APX001A and APX001 against *Rhizopus oryzae*, the most common cause of mucormycosis.

METHODS

- *Rhizopus delemar* 99-880 is a clinical strain isolated from a patient with rhinocerebral mucormycosis (2).
- The *in vitro* susceptibility of APX001A was evaluated using the Clinical Laboratory and Standards Institute (CLSI) M38-A2 method.
- ICR mice were immunosuppressed by cyclophosphamide (200 mg/kg) and cortisone acetate (500 mg/kg) on days -2, +3, and +8 relative to infection (3).
- Immunosuppressed mice were intratracheally infected with *R. oryzae* susceptible strain.
- For survival studies, treatment with oral APX001 given twice daily (bid) started 16 h post infection and continued through day +8. Treatment with once daily intravenous injection of LAmB (15 mg/kg) started 16 h post infection and continued through day +4.
- For tissue fungal burden, treatment with either drug started 8 h post infection and continued through day +3. Mice were sacrificed on day +4 and fungal burden in target organs was determined by qPCR (4).
- Placebo mice received vehicle 5% dextrose water given by oral gavage.
- Statistical analysis was carried out by the non-parametric Wilcoxon Rank Sum test for the tissue fungal burden and by Log Rand Sum test for the survival studies with *P* values of <0.05 being significant.

SUMMARY/CONCLUSIONS

- APX001A has *in vitro* activity against *R. delemar,* a leading cause of mucormycosis.
- In general, APX001 (bid) demonstrated efficacy in prolonging survival of neutropenic mice infected with *R. delemar*.
- Lower doses of 52 or 104 mg/kg (bid) of APX001, prolonged survival of neutropenic mice with pulmonary mucormycosis due to *R. delemar* at a level similar to high dose LAmB.
- At 156 mg/kg (bid), APX001 did not enhance survival of mice when compared to Placebo. These mice also looked unwell.
- All treatments resulted in significant reduction (~ 1 log) in Lungs CFU, while only APX001A at 105, and 156 mg/kg (bid) and LAmB resulted in significant reduction of brain fungal burden
- The lack of survival efficacy seen in mice treated with APX001 at 156 mg/kg (bid), while the same dose is able to reduce organs CFU suggests potential mouse-specific toxicity with higher doses of this drug.
- These data strongly support further development of APX001 in the treatment of mucormycosis.

REFERENCES

1. Ribes *et al.* Clin Microbiol Rev. 2000;13:236-301.
2. Ibrahim *et al.* Antimicriob. Agents Chemother 2003:47:3343-4.
3. Luo *et al.* Antimicriob. Agents Chemother. 2013;57:3340-7.
4. Ibrahim *et al.* Antimicrob. Agents Chemother. 2005;49:721-7

RESULTS

Table 1. *Rhizopus delemar* is susceptible to APX001A.

Species	MIC (50% inhibition)	MIC (100% inhibition)
R. delemar 99-880	0.25 µg/mL	8 µg/mL

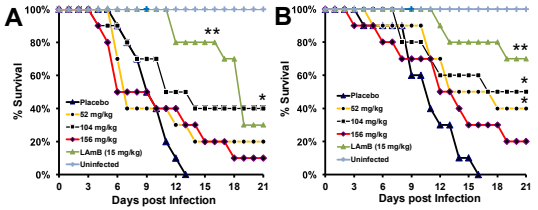

Figure 1. Survival of mice treated for 8 days or 4 days with APX001 or LAmB, respectively. (A) Experiment 1; (B) Experiment 2. N= 10 per group in each experiment. APX001 was tested at the doses of 52, 104, and 156 mg/kg bid, while LAmB was given once daily at 15 mg/kg. *P < 0.05 vs. placebo and ** P< 0.02 vs. placebo or APX001 at 104 mg/kg.

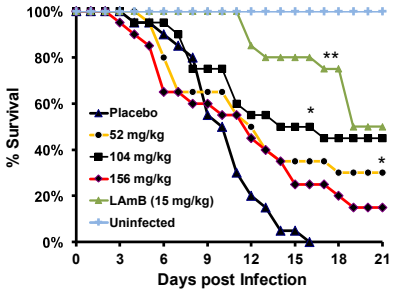

Figure 2. Combined survival data of mice treated for 8 days or 4 days with APX001 or LAmB, respectively. N= 20 per group. *P < 0.05 vs. placebo; **P <0.03 vs. placebo, 40. or 120 mg/kg.

ACKNOWLEDGEMENTS

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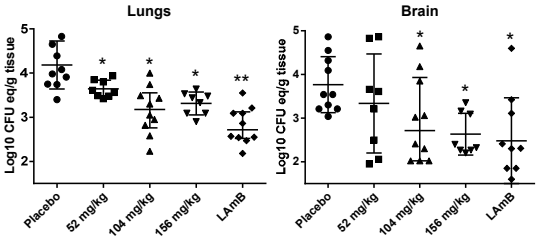

Figure 3. Experiment 1. Tissue fungal burden in lungs and brains of mice treated with APX001 or LAmB. Mice (N=10 per group) were infected intratracheally and then treated with APX001 (52, 104 or 156 mg/kg) or LAmB (15 mg/kg) from Day 0 -Day +3. * P <0.05 vs. placebo mice; **P <0.05 vs. placebo, 40 mg/kg or 120 mg/kg APX001.

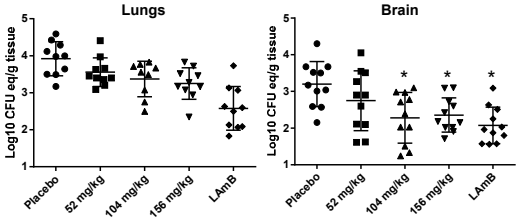

Figure 4. Experiment 2. Tissue fungal burden in lungs and brains of mice treated with APX001 or LAmB. Mice (N=10 per group) were infected intratracheally and then treated with APX001 (52, 104 or 156 mg/kg) or LAmB (15 mg/kg) from Day 0 -Day +3. * P <0.05 vs. placebo mice.

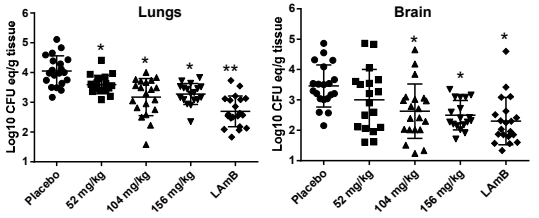

Figure 5. Combined data of tissue fungal burden in lungs and brains of mice treated with APX001 or LAmB. Mice (N=20 per group) were infected intratracheally and then treated with APX001 (52, 104 or 156 mg/kg) or LAmB (15 mg/kg) from Day 0 -Day +3. * P <0.05 vs. placebo mice; **P <0.05 vs. all other treatments.