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APX001 is Effective in the Treatment of Murine Coccidioidomycosis

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ABSTRACT

Background: Coccidioidomycosis (San Joaquin Valley Fever) is an infection caused by *Coccidioides* spp., which is endemic to areas in the Southwestern US and adjacent areas in Mexico. There are no FDA approved drugs for this infection but amphotericin B and most azole drugs show activity *in vitro* and *in vivo* and are widely used. However, infections that disseminate outside the lung require treatment for years or even a life-time, so there is a need for new, potent, well tolerated, orally available drugs for this disease. The prodrug APX001 is a first-in-class, I.V. and orally available broad-spectrum antifungal agent in clinical development for the treatment of life-threatening invasive fungal infections. The active moiety APX001A inhibits Gwt1, an enzyme required for cell wall localization of glycosylphosphatidylinositol (GPI) anchored mannoproteins in fungi (1).

Methods: We evaluated the activity of APX001A *in vitro* against spores from 10 strains of *Coccidioides*, including both *C. immitis* and *C. posadasii*, using a CLSI-recommended micro-broth dilution assay to determine the minimal effective concentration (MEC). The efficacy of APX001 was also evaluated in a pulmonary coccidioidomycosis model in which 16 C57BL/6 mice were infected intra-nasally with 200 spores of *C. immitis* RS. Treatment was initiated one week after infection, by which time all spores had converted to spherules, the invasive form of the fungus. Eight mice received either APX001 (26 mg/kg) 2 hours after the administration of 1-aminobenzotriazole (ABT), a pan-cytochrome P450 inhibitor (2). Control received only ABT in buffer. Mice were weighed before and after the experiment. At necropsy, one day after the last dose, lungs and spleens were cultured quantitatively.

Results: The MEC values of APX001A ranged from 0.001- 0.016 µg/mL. Treatment of mice with APX001 or APX2097 reduced $\log_{10}$ CFU in the lung by >2.5 fold ($P < 0.001$) versus untreated control and completely prevented dissemination to the spleen. No weight loss was observed in uninfected, treated mice or in infected and treated mice.

Conclusions: Although there are no accepted standards for interpreting MEC values for *Coccidioides,* the range of MEC was very low, indicating that APX001A and analogs were highly active against the filamentous form of *Coccidioides in vitro.* APX001 and APX2097 were very effective *in vivo* against spherules in a pneumonia/disseminated infection mouse model, making this a promising new candidate therapy for this recalcitrant infection and prolonged survival significantly longer than fluconazole.

METHODS

Isolates. All strains were originally clinical isolates. However, *C. immitis* RS, *C. posadasii* Silvera, and *C. posadasii* C735 have been passaged for years in different laboratories.

***In vitro* susceptibility testing.** Drug susceptibility tests were performed using a micro-broth dilution method according to the Clinical and Laboratory Standard Institute (CSLI) M38-A2 2008. MEC values were evaluated for APX compounds whereas 100% inhibition was evaluated for fluconazole.

Mice. C57Bl/6J female mice were purchased from Jackson Laboratory at-8 weeks of age and infected one week after arrival.

Infections and treatment. Mice were anesthetized and then infected with ~200 spores in 20ul sterile saline. Treatment was initiated seven days post infection and continued for 5 days: fluconazole, 25 mg/kg twice daily by gavage, or an APX drug at 26 mg/kg daily. APX001 has a short t½ in mice (2) so APX groups were pretreated with 50mg/kg of the pan-CYP inhibitor 1-aminobenzotriazole (ABT) 2 hours prior to administering an APX drug in order to increase the half-life and exposure of the compounds (3). One day after treatment ended (Day 13 post infection), mice were sacrificed to do quantitative cultures of lungs and spleens.

Compounds tested: APX001A is the active moiety of the N-phosphonooxymethyl prodrug APX001. Similarly, APX2020 and APX2041 are the active moieties of APX2097 and APX2104, respectively.

Statistics. Geometric mean ±1 SEM CFU/organ were calculated and compared using paired t test. For multiple groups we used Dunnett's ANOVA test. Kaplan-Meier survival curves were analyzed by log rank (Prism 7).

RESULTS

Fig 1. Structures of Gwt1 inhibitors

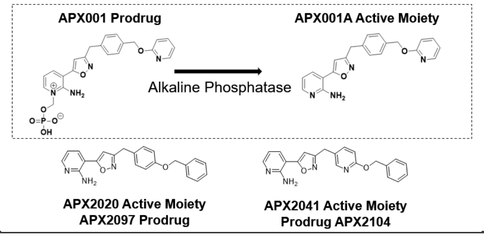


Table 1. *In vitro* susceptibility

Coccidioides Isolate	Source	Microbiological Activity			
		MEC (ng/mL)			MIC (ng/mL)
		APX001A	APX2020	APX2041	Posaconazole
C. immitis RS	Lab	2-4	2-4	2-4	60-125
C. immitis B2358	CDC	4	4	0.125	16
C. immitis F40	Clinical	4	2	1	125
C. immitis F1	Clinical	2	1	1	125
C. immitis UCSD2	Clinical	1	1	0.25	125
C. posadasii F6	Clinical	16	4	1	125
C. posadasii Silvera	Lab	8	8	4	30
C. posadasii F5	Clinical	8	4	1	16
C. posadasii C735	Lab	4	2	2	60-125
C. posadasii D2A	Clinical	4	2	1	30

Fig 2. ABT has no antifungal activity *in vivo* and no affect on weight loss after infection

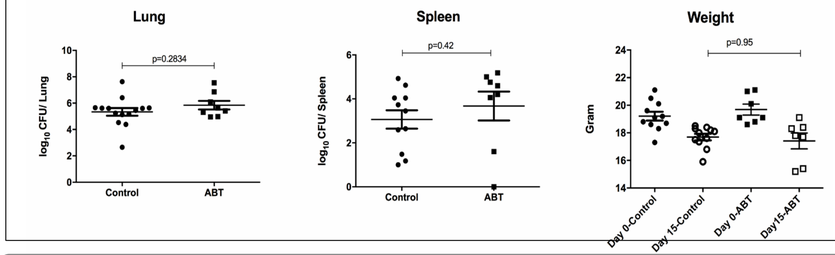
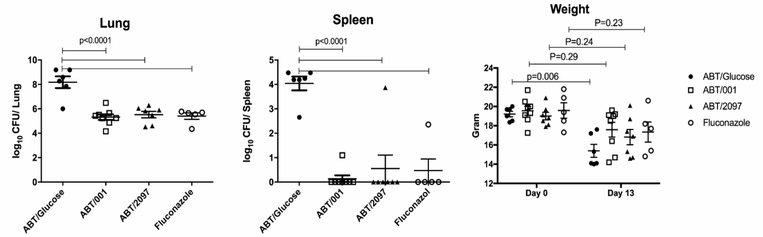
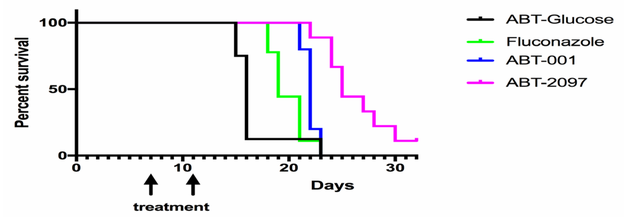


Fig 3. *In vivo* efficacy: CFU reduction and Body Weight



- Equivalent decreases in lung and spleen CFU were observed after treatment with APX001, APX2097 and fluconazole
- Weight loss was significantly greater for the untreated mice. No significant differences were observed among the treatment groups.

Fig 4. *In vivo* efficacy: survival



- Mice were treated for 5 days (arrows) and then observed until death
- All treatments prolonged survival but the APX treated mice demonstrated significantly longer survival than untreated or fluconazole treated mice (P=< 001).

CONCLUSIONS

- APX001A and analogs are highly active against *C. immitis* and *C. posadasii*
- APX001, APX2097 and fluconazole inhibited multiplication of *C. immitis* in mice
- All 3 treatment groups had the same geometric mean numbers of CFU at the end of the treatment period, but the Gwt1 inhibitors were more effective at prolonging survival.

ACKNOWLEDGEMENTS AND REFERENCES

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