Fosmanogepix (APX001) is Effective in an Immunosuppressed Mouse Model of Fusariosis

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ABSTRACT

Background: Fusariosis has high mortality rates. Owing to its rarity, comparative clinical trials are hard to perform. Animal models are an appropriate complementary avenue for evaluating antifungal therapy. Thus fosmanogepix (APX001) was evaluated in an immunosuppressed murine model of hematogenously disseminated fusariosis.

Materials/methods: The minimum effective concentration (MEC) of manogepix (MGX, APX001A, the active moiety of fosmanogepix) was determined against a *Fusarium solani* clinical isolate using CLSI M38 methodology. ICR mice were immunosuppressed with cyclophosphamide and cortisone acetate on days -2, and +3, relative to intravenous infection with 8.1×10^2 cells of *F. solani*. For survival studies, treatment with placebo (diluent control), fosmanogepix (78, or 104 mg/kg, po, doses which achieve exposures anticipated to provide efficacy in clinical trials), liposomal amphotericin B (L-AMB, 15 mg/kg, iv), or voriconazole (VORI, 40 mg/kg, po) began 16 h post-infection and continued for 8 days for fosmanogepix or VORI and 4 days for L-AMB. To extend the half-life of fosmanogepix, mice were administered 50 mg/kg of the cytochrome P450 inhibitor 1-aminobenzotriazole (ABT) 2 h prior to fosmanogepix administration. Similarly, grapefruit juice (50%) was added in the drinking water for VORI-treated mice. To assess tissue fungal burden, mice were sacrificed on Day +4 and organs processed for conidial equivalent (CE) by qPCR.

Results: The manogepix MEC value for the tested *F. solani* strain was 0.03 µg/mL. Treatment with fosmanogepix or L-AMB enhanced median survival time vs. placebo (12, and 10 days for 78, and 104 mg/kg of fosmanogepix, respectively vs. 10 days for L-AMB treatment, vs. 8 or 7 days for VORI or placebo, respectively, $P<0.01$). Further, fosmanogepix and L-AMB treatments equally enhanced overall survival by day 21 when the experiment was terminated (40% for L-AMB or fosmanogepix at 78 mg/kg, and 20% for fosmanogepix at 104 mg/kg, vs. 0% for placebo or VORI treatment). Fosmanogepix or L-AMB treatments, but not VORI, resulted in ~ 2-3 log reduction in kidney, and brain CE vs. placebo

Conclusions: Fosmanogepix was as effective as L-AMB in protecting immunosuppressed mice from fusariosis. Continued investigation of fosmanogepix as a novel antifungal agent against fusariosis is warranted.

INTRODUCTION/AIMS

- *Fusarium* is a major cause of superficial infections of onychomycosis and keratitis in immunocompetent individuals and serious hematogenously disseminated infections in severely immunocompromised patients, such as patients with hematologic malignancies, that are often associated with poor outcome (1, 2).
- Despite current treatments options, systemic fusarioses are associated with high mortality rates of 80-100%.
- Due to the rarity of the disease, clinical trials for fusariosis are problematic and the optimal antifungal therapy is unclear.
- Fosmanogepix (APX001) is a first-in-class small molecule antifungal that is currently in clinical development for the treatment of invasive fungal infections (3).
- Fosmanogepix is an N-phosphonooxymethyl prodrug which is rapidly and completely metabolized by systemic phosphatases to the active moiety, manogepix (APX001A) (4).
- Manogepix targets the highly conserved fungal enzyme Gwt1, which catalyzes an early step in glycosylphosphatidyl inositol (GPI)-anchor biosynthesis (5).
- Here, we assessed the *in vitro* and *in vivo* activity of manogepix/fosmanogepix against *F. solani,* respectively.

REFERENCES

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METHODS

- ***In vitro* testing**. The *in vitro* susceptibility of MGX, VORI and L-AMB against agents of fusariosis was evaluated using the Clinical Laboratory and Standards Institute (CLSI) M38-A2 method. MEC values were determined as per the echinocandins.
- **Immunosuppression.** Male ICR mice were immunosuppressed with cyclophosphamide (200 mg/kg) and cortisone acetate (500 mg/kg) on days -2, and +3, relative to infection.
- **Infection.** Immunosuppressed mice were infected with 8.1×10^2 cells of *F. solani* 95-2478 by tail vein injection.
- **Treatment.** For survival studies, treatment with placebo (diluent control), fosmanogepix (78 or 104 mg/kg, PO), L-AMB, (15 mg/kg, IV), or VORI (40 mg/kg, PO) began 16 h post-infection and continued for 8 days for fosmanogepix or VORI, and 4 days for L-AMB. To enhance the half-life of VORI, grapefruit juice (Ocean Spray) was added in the drinking water to a final concentration of 50%. To extend the half-life of fosmanogepix, mice were administered 50 mg/kg of the cytochrome P450 inhibitor 1-aminobenzotriazole (ABT) 2 h prior to fosmanogepix administration (6).
- **Efficacy endpoints.** Survival of mice for 21 days and tissue fungal burden (by qPCR) and histopathological examination were assessed on Day 4 post infection.
- **Statistical Analysis.** The nonparametric log-rank test was used to determine differences in survival times. Differences in lung, kidney and brain CE were compared by the nonparametric Wilcoxon rank-sum test. A *P* value < 0.05 was considered significant.

SUMMARY/CONCLUSIONS

- Manogepix has a potent *in vitro* activity against the *F. solani* clinical isolated tested.
- Fosmanogepix was as effective as L-AMB in prolonging median survival time and improving overall survival of mice infected with disseminated fusariosis.
- Fosmanogepix prodrug significantly reduced tissue fungal burden in kidney and brain when compared to placebo-treated mice and was comparable to L-AMB treatment. These effects were at a dose in mice that is equivalent to the projected dose in humans.
- Histopathological examination of target organs corroborated the findings of tissue burden with mice treated with fosmanogepix showing reduced or no fungal abscesses.
- Continued investigation of fosmanogepix as a novel antifungal drug against fusarioosis is warranted

ACKNOWLEDGEMENTS

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RESULTS

Figure 1. Chemical structures of APX001 and APX001A

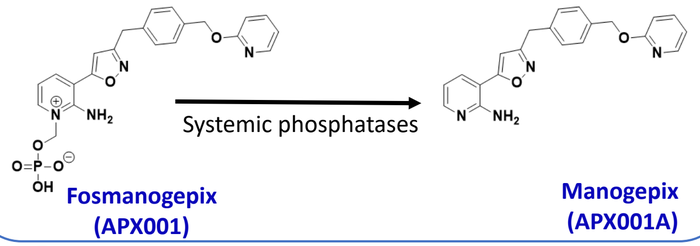
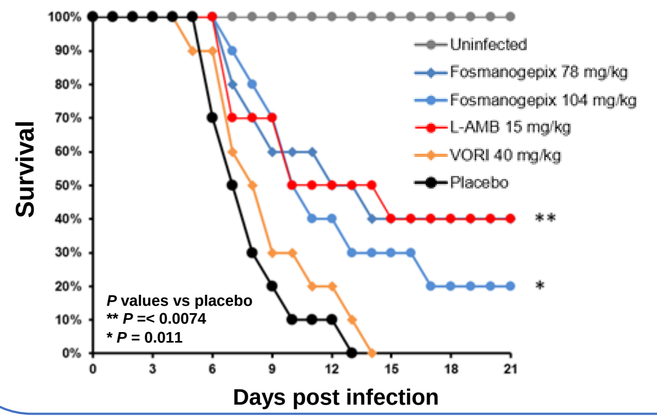


Table 1: Minimum effective concentration (MEC) and MIC against *F. solani*

Drugs	MEC (µg/ml)	MIC (µg/ml)
Manogepix	0.03	
Voriconazole		0.4
L-AMB		0.8

Figure 2. Fosmangepix prolonged survival of immunosuppressed mice (n=10/group) infected with *F. solani*



Dose	tAUC	
Placebo		●
Fosmanogepix 78 mg/kg + ABT	207	■
Fosmanogepix 104 mg/kg + ABT	282	▲
L-AMB 15 mg/kg		○

Treatment	Median survival time (days)	*P* value (vs. Placebo)
Placebo	7	
Fosmanogepix 78mg/kg (ABT)	12	0.0074
Fosmanogepix 104mg/kg (ABT)	10	0.0110
VORI 40mg/kg BID	8	0.3071
L-AMB 10mg/kg	10	0.0047

Figure 3. Fosmanogepix demonstrated statistically significant reductions vs. placebo-treated mice that were equivalent to, L-AMB treatment.

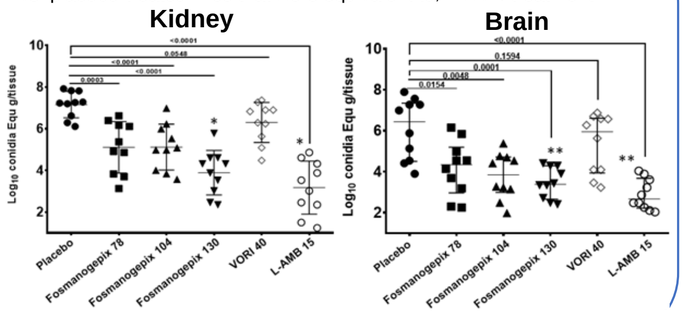


Figure 4. Fosmanogepix showed less hyphae in kidney tissue vs. placebo-treated mice that were equivalent to L-AMB treatment.