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A Novel Encochleated Atovaquone Formulation is Active in a Murine Model of *Pneumocystis* Pneumonia

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Background

- *Pneumocystis spp.* are yeast-like fungi that can cause *Pneumocystis* pneumonia (PCP) in immune-compromised hosts. Over the past two decades, the incidence of PCP in patients with HIV in the United States has declined, mostly due to the widespread use of prophylaxis in patients who are susceptible, as well as the development of Highly Active Anti-Retroviral Therapy (HAART). However, for those who develop PCP, the associated mortality in the United States remains relatively stable at 10-15%.
- Trimethoprim/sulfamethoxazole (TMP-SMX) is the standard of care treatment for *pneumocystis* pneumonia (PCP), however, it suffers a high rate of adverse events (20-85%) including: Rash (can be severe and progression to Stevens-Johnson syndrome and toxic epidermal necrolysis are possible), fever, hepatotoxicity, and bone marrow suppression.
- Subsequently, up to 36% of patients experience toxicity that leads to discontinuation of TMP-SMX
- Atovaquone is alternative agent for treatment and prophylaxis of PCP in patients who are unable to tolerate trimethoprim/sulfamethoxazole
- The current commercially available formulations of atovaquone suffer from limitations of saturable absorption/pharmacokinetics and poor tolerability due to complaints of poor taste/palatability, nausea, diarrhea, rash, headache and transaminase elevations , with up to 20% discontinuation rates due to these adverse events.
- Cochleates are a stable, orally bioavailable, lipid-crystal, nanoparticle drug delivery platform composed primarily of phosphatidylserine and calcium with a lack of a internal aqueous space.
- In a previous study of a different formulation of CATQ, promising efficacy was observed, however a toxicity of papillary necrosis developed in mice treated with CATQ plus anidulafungin.
- We sought to evaluate the PK, efficacy, and toxicity of a novel a novel lipid-crystal nanoparticle formulation of encochleated atovaquone (CATQ) in a murine model of *pneumocystis* pneumonia.

Disclosures & Funding

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Study Design and Methods

Murine infection model:

- Mice immunosuppressed with dexamethasone and infected with *P. murina* (infected by cohousing with a *P. murina* infected mouse and immunosuppressed by addition of 4 µg/ml of Dexamethasone to the drinking water for 5 weeks) were utilized in all 3 studies study.
- In all studies, all drugs were administered via oral gavage.

Efficacy study 1:

- Infected mice (n = 8 each) were treated daily for 14 or 21 days with either: commercial atovaquone suspension (Mepron) 100 mg/kg, trimethoprim/sulfamethoxazole (TMP/SMX) 50/250 mg/kg, or CATQ dosed at 25, 50, 100, or 200 mg/kg.
- Measurements of survival, asci and nuclei counts were used to determine efficacy compared to controls (empty cochleates).

Efficacy/toxicity study 2:

- Infected mice (n= 8 each) received 14 days of treatment with either: anidulafungin 1 mg/kg TIW; Mepron 100 mg/kg/day; CATQ 100 mg/kg/day; empty cochleates; empty cochleates + Mepron; anidulafungin + empty cochleates; anidulafungin + Mepron; anidulafungin + empty cochleates + Mepron; anidulafungin + CATQ.
- In addition to evaluation of efficacy by organism burden, tissue homogenates of lungs, liver, spleen, kidneys, and brain were collected and processed for histology and pathologic assessment.
- Comparisons between groups were performed by one-way analysis of variance (ANOVA) followed by the Newman-Kuhls multiple comparison test and by the two-tailed *t* test for single time points when appropriate. Significance is accepted when the P value was<0.05. (GraphPad Software for Science, San Diego, CA).

PK study:

- Infected mice received CATQ at 100 mg/kg via oral gavage.
- Three mice from each dose group were sacrificed at ten time points (0 (baseline), 2, 4, 8, 12, 24, 48, 72, and 96 hrs for collection of blood and lung tissue. Blood was processed to plasma and lung tissue was homogenized for quantification of atovaquone via a liquid chromatography-tandem mass spectrometry assay.

Conclusion

- CATQ represents a viable potential therapeutic candidate for treatment and prophylaxis of PCP with further development warranted.

Results

Efficacy Study 1:

- There was a significant reduction in both nuclei and asci counts in all treatment groups versus the negative control (C/S) at day 14 and day 21 (Figure 1a).
- There was no statistical difference between the asci counts of the 200 mg/kg eATQ dose versus the positive control TMP/SMX at either timepoint.
- There was a significant reduction in both nuclei and asci counts between the 100 mg/kg eATQ dose and the 100 mg/kg Mepron dose at day 14.
- There was a consistent dose response between the eATQ doses at both timepoints.
- All treatment groups except the 25 mg/kg eATQ dose showed a statistically significant improvement in survival versus the C/S group at day 14 . All treatment groups except the 50 and 200 mg/kg eATQ groups showed significant improvement in survival versus the C/S group at day 21. It should be noted that 2 mice in the 50 mg/kg eATQ group and 1 mouse in the 200 mg/kg eATQ group died at days 1 and 2 for the day 21 timepoint, possibly due to the immune suppression regimen.
- No overt toxicity was observed during the study.

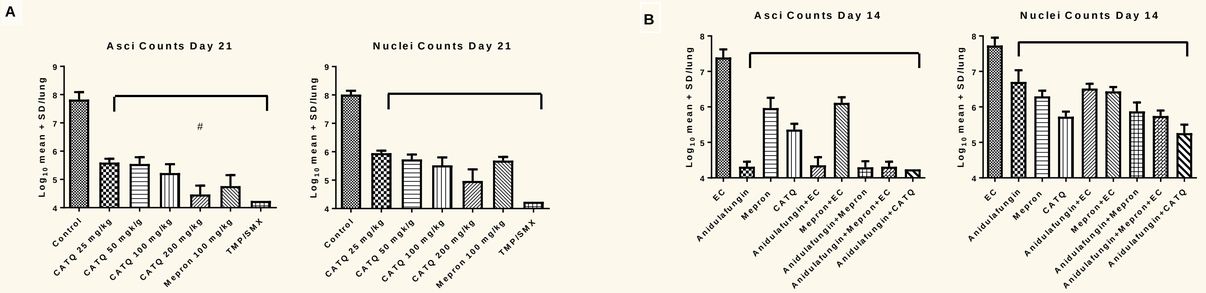

Figure 1. (A) Log_{10} mean nuclei and asci counts after 21 days of treatment. Bracket denotes statistical significant difference between treatment groups and control group. # denotes no statistical significant difference between treatment group and TMP/SMX. **(B)** Log_{10} mean nuclei and asci counts after 14 days of treatment. Bracket denotes statistical significant difference between treatment groups and empty cochleate (EC) control group Significance set at a P value $\leq$ 0.05.

Efficacy/toxicity Study 2:

- There was a significant reduction in both nuclei and asci counts in all treatment groups versus the negative control (C/S) at day 14 (Figure 1b).
- There was a significant reduction in both nuclei and asci counts between the eATQ alone dose versus the Mepron alone dose.
- There was a significant reduction in nuclei counts between the anidulafungin + eATQ dose versus the eATQ alone dose.
- There were no significant differences in survival between any groups. All mice in all groups survived until the day 14 sacrificed except one mouse in Group 4, which was found dead on day 14.
- There were no abnormal gross observations at necropsy or histopathology of kidney, liver, spleen, and brain tissue observed.
- There were differences noted in the lung between groups in regard to organism involvement: The combination of anidulafungin with either the encochleated atovaquone or the empty encochleates has significantly reduced burdens, suggesting this combination had increased efficacy after 14 days of treatment. All treatment groups had reduced inflammation when compared to the untreated mice, likely due to the decreased organism burdens and decreased Beta-glucan in the anidulafungin groups. The only group to have reduced consolidation was the anidulafungin + empty encochleates.

PK Study:

- CATQ demonostrated a favorable PK distribution profile, with a half-life ~13 hours in plasma and ~50 hours in lungs and detectable levels were observed in both plasma and lung through 96 hrs post-dose.