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Efficacy of Oral APX001 in a Murine Model of Cryptococcal Meningitis

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Introduction and Objectives

Cryptococcal meningitis kills about 181,000 people yearly, mainly in sub Saharan Africa. Standard of care treatment is a combination of IV amphotericin B plus oral 5-FC. APX001 (Amplyx Pharmaceuticals) is a first-in-class intravenous and orally available prodrug of APX001A, the active antifungal moiety. APX001A inhibits Gwt1, a fungal protein involved in glycosylphosphatidylinositol anchor biosynthesis, but does not affect the human homolog PIG-W. Activity for APX001 (APX) has been reported against invasive candidiasis, aspergillosis, fusariosis, and mucormycosis. In this study we evaluated the potential efficacy of APX001, alone and in combination with fluconazole (FCN), in a murine model of cryptococcal meningitis.

Materials and Methods

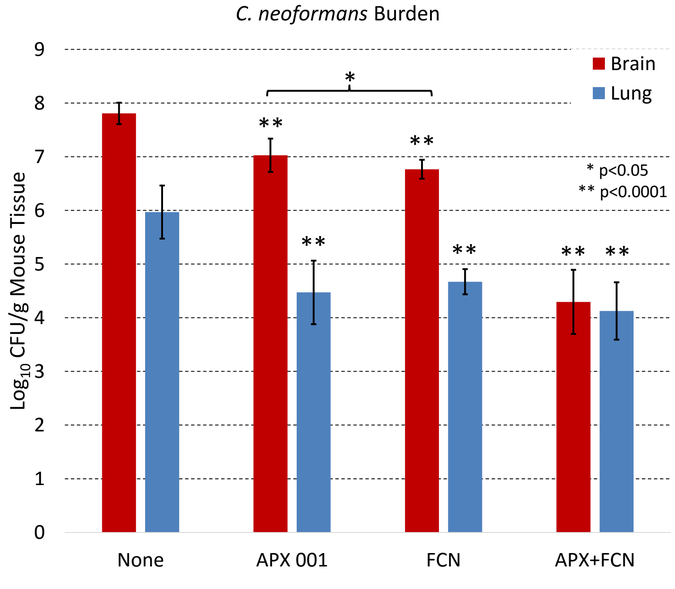
Cryptococcus neoformans strain H99 was grown in YPD broth at 30^{0}C on a shaker (220 rpm) for 24 hours, centrifuged (1980 rcf) and washed twice in PBS, resuspended in PBS, and quantified by hemacytometric count. CD-1 male mice were infected with 5.9 x 10^4 colony forming units (CFU) per mouse via lateral tail vein injection of 100µL. Mice were weighed and treatment was started immediately after infection. APX001 was administered by oral gavage at a dose of 390 mg/kg thrice daily, roughly eight hours apart. Fluconazole (2mg/mL, Sagent Pharmaceuticals, Inc.) was given at a dose of 80 mg/kg/day intraperitoneally. Mice were assigned to four groups, ten mice per group, consisting of treatment with fluconazole (FCN), treatment with APX001, treatment with both drugs in combination, or no treatment. Treatments were given for seven days. Mice were weighed daily and observed for acute and chronic adverse symptoms.

Mice were sacrificed on day 8, and brain and left lung were homogenized and cultured for quantitative determination of tissue burden (CFU per gram of tissue). Tissues were homogenized for 25 seconds in 1 mL phosphate buffered saline using two 6.5mm steel beads and a Mini-Beadbeater 16 (Biospec Products, Inc., Bartlesville, OK), and serially diluted in ten-fold steps. Aliquots (100µL) of homogenate were plated and incubated for 3-7 days at 37^{0}C. Data were $\log_{10}$ transformed and evaluated using the unpaired t-test (GraphPad Software, Inc., La Jolla, CA).

Results

In brain, the burden of *C. neoformans* in mice receiving combined therapy was 3.52 log lower than in untreated control mice. The burden in mice receiving APX001 alone was 0.78 log lower than in untreated mice. The burden in mice receiving FCN alone was 1.04 log lower than in untreated mice.

In lung, the burden in mice receiving combined therapy was 1.84 log lower than in untreated control mice. The tissue burden for mice receiving APX001 alone was 1.58 log lower than in untreated mice. The tissue burden in mice receiving FCN alone was 1.3 log lower than untreated mice.



Brain and lung tissue burden ($\log_{10}$ CFU per gram tissue weight)

		Brain		Lung	
Group	No. of mice	Mean Log CFU/g	Std Dev Log CFU/g	Mean Log CFU/g	Std Dev Log CFU/g
Untreated	9	7.81	0.19	5.97	0.47
APX	9	7.03	0.29	4.47	0.56
FCN	10	6.77	0.17	4.67	0.22
APX+FCN	9	4.29	0.56	4.13	0.49

Conclusions

- Activity in murine brain
  - Combined therapy of APX001 with FCN significantly inhibited growth of *C. neoformans* H99 compared to untreated control mice ($p<0.0001$), and was significantly more active than monotherapy with APX001 or FCN ($p<0.0001$ and $p<0.0003$, respectively).
  - APX001 and FCN each, alone, significantly inhibited growth of *C. neoformans* H99 in brain tissue compared to untreated control mice ($p<0.0001$).
- Activity in murine lung
  - Combined therapy of APX001 with FCN performed somewhat better than FCN alone ($p=0.0297$), but no better than APX001 alone ($p=0.2500$).
  - APX001 and FCN each, alone, significantly inhibited growth of *C. neoformans* H99 in lung tissue compared to untreated control mice ($p<0.0001$).
- Significant potentiation of APX001 in combination with FCN in this model with *C. neoformans* H99 was observed within the brain, and further investigation is warranted to determine whether APX001 in combination with FCN has potential to be an effective all oral regimen for treating cryptococcal meningitis.

Acknowledgements and Disclosure

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