Impact of 5-LO inhibitor, Zileuton, on the efficacy if antifungal therapy against *Cryptococcus in vitro*

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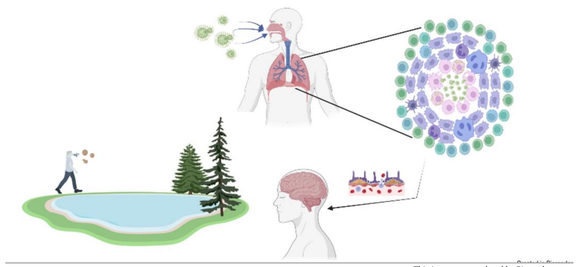
Abstract

Individuals with severe immunocompromised conditions face a 10% to 25% mortality rate when infected with *Cryptococcus* fungi. To better understand *Cryptococcus* pathogenesis and explore strategies to mitigate infection severity, research has identified leukotrienes (LTs) as critical lipid modulators exploited by fungi. LTs are derived from arachidonic acid through the enzymatic activity of 5-lipoxygenase (5-LO). Studies in our lab have shown that mice deficient in 5-LO exhibit reduced disease severity when exposed to *Cryptococcus deneoformans* strain 52D. In contrast, wild-type (WT) mice infected with the same strain developed meningoencephalitis, leading to a higher mortality rate. These findings suggest modulating host 5-LO activity could be a therapeutic strategy for reducing *Cryptococcus* infection severity. Our lab demonstrated that treating WT mice with zileuton, a 5-LO inhibitor, increased survival rates. To further evaluate the potential of 5-LO inhibitors like zileuton in alleviating brain-related symptoms during infection, this project examines the drug interaction between zileuton and commonly used antifungal treatments—amphotericin B, 5-flucytosine, and fluconazole—to determine any impact on antifungal efficacy. Our preliminary results indicate that zileuton does not interfere with fluconazole and amphotericin B activity, suggesting zileuton could be used with antifungal drugs to ameliorate the symptoms during *Cryptococcus* infections.

Introduction

Cryptococcus neoformans is a widespread fungal pathogen that poses a threat to immunocompromised individuals, leading to pneumonia and fatal meningoencephalitis.



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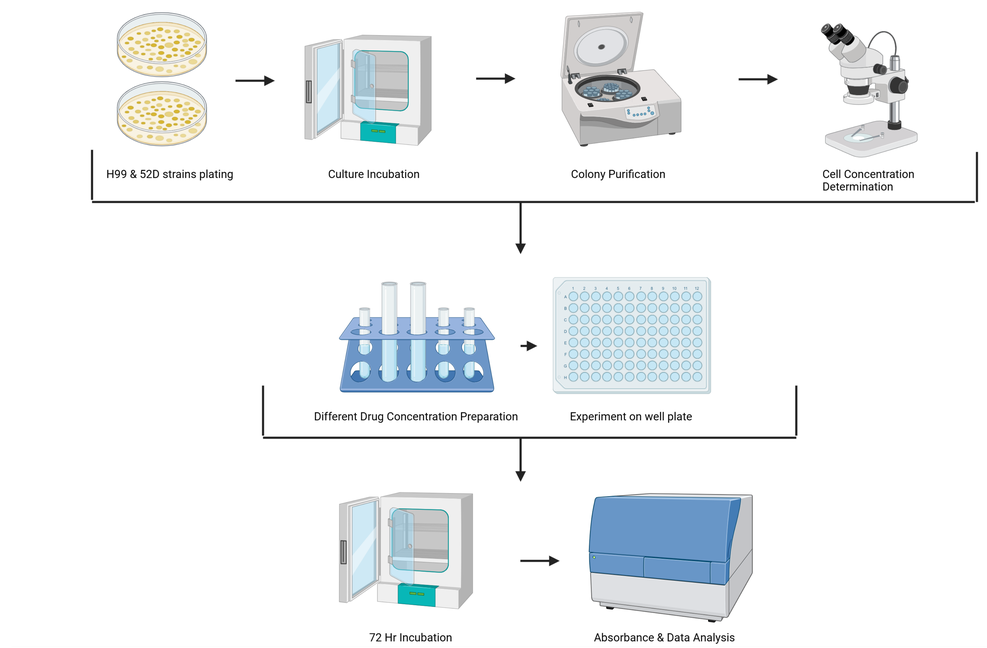
Currently, three classes of antifungals—polyenes, flucytosine, and azoles—are often used to treat cryptococcal infections. They are often used in a three-part therapeutic strategy to combat disseminated infection. But in cases of C-IRIS, the exuberant immune response often causes greater damage; for this reason, controlling the immune response to give time for these antifungals to work is key.

Material and methods



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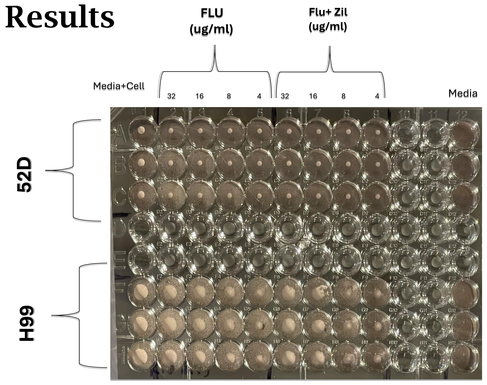
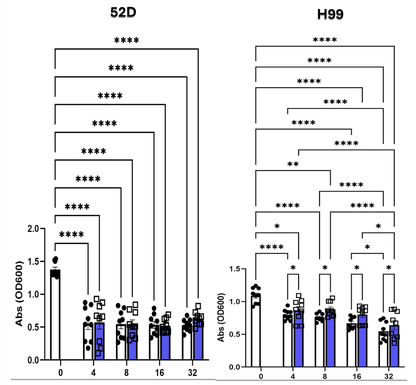
Results



Using a standardized testing method, we observe differences in the growth **of both *C. neoformans* H99 and *C. deneoformans* 52D when treated with Fluconazole or Amphotericin B at different concentrations, and Zileuton was kept constant concentration**

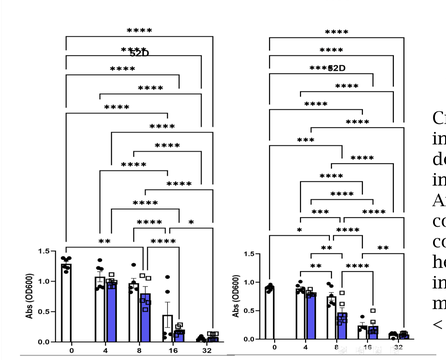
Figure 1. Susceptibility of *Cryptococcus* against Fluconazole and constant Zileuton concentration

Figure 2. Zileuton did not affect the fungistatic activity of fluconazole



Cryptococcal cells were grown and incubated under the conditions described by NCCLS with either increasing concentrations of fluconazole or fluconazole plus zileuton, where the concentration of zileuton was kept constant (add concentration of zileuton here). Cells were grown at 37˚C in cRPMI in 5% CO_2 for 72 h, and absorbance was measured at 48 and 72h. (*p < 0.05, **p < 0.01, ***p < 0.001, ***p < 0.0001)

Figure 3. Zileuton did not affect the fungicidal activity of Amphotericin B (AmB)



Cryptococcal cells were grown and incubated under the conditions described by NCCLS with either increasing concentrations of AmB or AmB plus zileuton, where the concentration of zileuton was kept constant (add concentration of zileuton here). Cells were grown at 37˚C in cRPMI in 5% CO_2 for 72 h, and absorbance was measured at 48 and 72h. (*p < 0.05, **p < 0.01, ***p < 0.001, ***p < 0.0001)

Future directions and conclusions

- Both Fluconazole and AmB were able to halt the growth of the H99 and 52D strains when in combination with zileuton. Suggesting that zileuton is not interfering with the antifungal activity of these drugs.
- Next, we will test these antifungal drugs in combination, as used in patients, to evaluate the interaction of zileuton when used with more than one antifungal.

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