A Novel Therapeutic Approach for Cryptococcal Meningitis

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Introduction

Cryptococcal meningitis (CM) is a devastating opportunistic infection that primarily presents in immunocompromised patients. Human immunodeficiency virus (HIV) and corticosteroid induced immunosuppression in transplant patients are the primary risk factors for CM.[1] There are an estimated one million CM cases globally, with mortality rate estimates around 65%.[2]

CM is caused when *Cryptococcus neoformans*, a basidiomycete fungal pathogen, invades the central nervous system (CNS) and circulates around the brain and spinal cord in the subarachnoid space (SAS).[3,4] *C. neoformans* is a budding yeast that has a diameter ranging in size between 5-10 um, with an extracellular polysaccharide capsule that can reach a diameter upwards of 50-60um total. Cryptococcal cells can mechanically occlude arachnoid villi by shedding their polysaccharide antigen glucuronoxylomannan (GXM), commonly known as Cryptococcal antigen (CrAg).[5] Since the production rate of CSF is independent of intracranial pressure (ICP), this interruption in CSF flow puts infected patients at serious risk of developing elevated ICP and hydrocephalus.

Current treatment guidelines include two weeks of continuous intravenous administration of amphotericin B and flucytosine, and maintaining a fungicidal regimen for six months. However, poor drug penetration and high costs often limit the effectiveness of those therapies. The single most important factor impacting survival was shown to be rapid reduction in CSF organism in the first two weeks of infection.[6]

Hypothesis and Experimental Goals

We hypothesized that *C. neoformans* could be eliminated by mechanical filtration of the CSF (Neurapheresis™), directly reducing the burden of known pathogens in the CSF, lowering ICP and decreasing the occlusion of arachnoid villi. Using an infected rabbit model of CM, we designed and tested an experimental filtration system to cause a 1-2 log reduction in CSF-organism burden. Our experimental goals are outlined below.

1. Demonstrate in vitro reduction of *C. neoformans* in bench-top experiments
2. Demonstrate in vivo reduction of CSF-burden using validated CM rabbit model.
3. Transition towards translational study in human model of CM

In Vitro Methods

In vitro growth of C. neoformans:

A clinically isolated strain, H99, was grown for 24 hours in 5 ml YPD (yeast peptone dextrose) media at 30 C for cell proliferation. Transferred to 25 ml of Diluted Sabouraud media and incubated for 24 hours to induce capsule growth (Image 1).

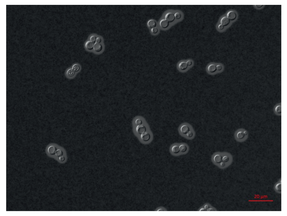

Image 1. H99 *C. neoformans* in vitro.

Bench Top Filtration:

H99 grown in capsule-inducing conditions and suspended in artificial CSF (aCSF) circulated through a closed loop system and passed through different pore size filters to evaluate clearance capacity. Optimal filter has maximal clearance and minimal max PSI.

Filter Selection:

Membrane (dead-end) filtration was selected because of low priming volume requirements. Polyethersulfone (PES) membrane was selected because of low protein binding capacity and hydrophilicity.

Surgical Procedure

- CM initiated in 10 NZW rabbits by steroid regimen and intracisternal injection of 0.3 mL of 108 CFU/mL of C. *neoformans*
- Test infected rabbit's ability to undergo 8-10 hour anesthesia and assisted respiration
- Cisternal cutdown and lumbar laminectomy used to gain access to SAS
- Threaded 2 Fr catheters into SAS and sealed to create closed-loop system (Image 2).
- CSF circulated via peristaltic Pump while CSF samples were obtained regularly throughout the procedure. Samples were later assessed for colony forming units (CFUs).

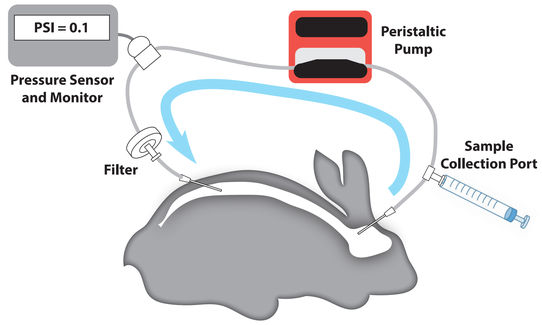

Image 2. In vivo testing setup

Results

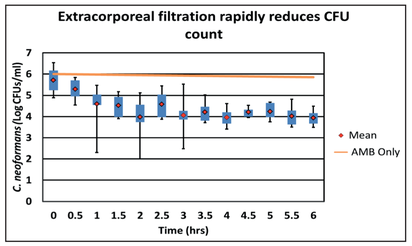

Figure 1. CSF CFU reduction over 6 hours of Neurapheresis. Filtration typically induced a 1-log reduction in CFU count after 1.5 hours and a 2 log reduction after 6 hours. AMB Only in rabbits and humans reduces CFUs by 0.62 and 0.42 log/day, respectively.[7,8]

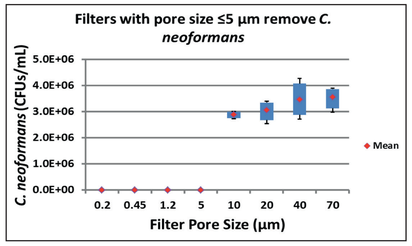

Figure 2. Post-filtration cell counts using various filter membrane pore sizes. Membrane pores ≤5 µm remove all CFUs from aCSF

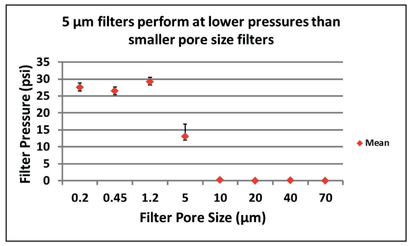

Figure 3. Maximum transmembrane pressures from various membrane pore-size filters. 5 µm filters provide maximum filtration with minimal pressure build-up.

Conclusion

We observed a 1-log (90%) reduction in CSF fungal CFUs within hours of cycling in all animals. In three animals, we observed a 2-log (99%) reduction in CFU burden over 6 hours. For comparison, recent studies have shown that the best systemic combination antifungal therapy in humans approaches 0.4-log yeast reductions per 24 hours.[8]

We conclude that Neurapheresis may provide a mechanical means to substantially reduce the *C. neoformans* CFU burden in the CSF and may serve as a novel adjunctive therapy for the treatment of CM and other CNS infections.

Future studies may look to incorporating combination drug therapy with amphotericin-B and flucytosine, as the Neurapheresis technique may have implications on perfusion of drug throughout the SAS. Additionally, filtration of the polysaccharide capsule could eliminate the occlusion of the arachnoid villi, and may address the hydrocephalus that is commonly seen in CM patients

Larger studies are needed to validate the technique and model, and explore its ability to remove smaller molecules like cytokines and antigens, infuse antifungals, and control ICP in the SAS.

Translational Applications

Testing is underway to translate this research to humans. A novel catheter-based extracorporeal filtration system has received FDA IDE approval for first in human evaluations and will soon begin clinical testing in a different patient population (Image 3). This system has demonstrated ability to remove both the *C. neoformans* organism and its polysaccharide capsule in vitro in experimental bench and animal studies.

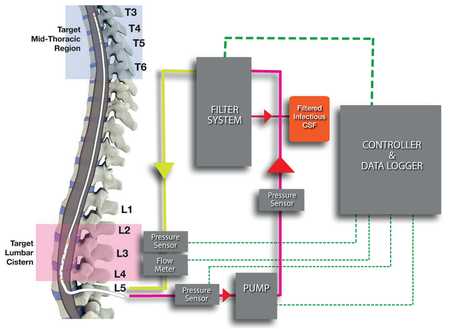

Image 3. Human system schematic

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