Background

Fungal meningitis (FM) is an infection of the meninges of the central nervous system that manifests from the dissemination of any major fungal pathogen into the subarachnoidal space (SAS) via the cerebrospinal fluid (CSF). Cryptococcal Meningitis (CM) is caused by Cryptococcus neoformans and is the most common cause of fungal meningitis in adults. Treatment for CM is based on an induction, consolidation, and maintenance approach with antifungals and is well-defined elsewhere, but is associated with continued high morbidity and mortality. Drug discovery programs are limited by poor penetration of the Blood Brain Barrier (BBB). Because of this, we developed an alternative catheter-based extracorporeal system (Neurapheresis™ Therapy) for the filtration of infected CSF. Here we describe the in vitro characterization of Neurapheresis™ Therapy as an alternative mechanical intervention for filtration of C. neoformans cells, polysaccharide antigen, and inflammatory mediators from infected CSF.

Methods

In Vitro Growth of C. neoformans

H99, a clinical strain of C. neoformans, was grown overnight in 5mL YPD (yeast peptone dextrose) at 30°C for cell proliferation. Cells were the transferred to 25mL of diluted Saboraud/MOPS media and incubated for 24 hours at 37 °C to induce capsular growth. Cells were diluted to clinically relevant concentrations (1x10^6 and 1x10^7 cells/mL) for experiments.

Benchtop Filtration

Cells were diluted to clinically relevant concentrations in 150 mL of Saboraud/MOPS and passed through the closed-loop Neurapheresis system equipped with either 100kDa or 5kDa tangential flow filters (TFF). The setup is demonstrated in Figure 1 and consists of an “inlet” line leading from the sample reservoir to the filtration unit, the filter assembly itself, a device data recorder, a waste line for concentrated removal of organism, and an “outlet” line with a split allowing both clean sampling of the “permeate” and feed back into the sample reservoir. Samples were taken every full CSF volume filtration cycle (150 mL) for quantification of yeast load, antigen, and cytokines. Infected human CSF was used to obtain cytokine data.

Assays

• Colony Forming Units (CFUs) were determined to define organism concentration.
• Cryptococcal Antigen was quantified using a Lateral Flow Assay (LFA by IMMY)
• Cytokines were quantified by a Luminex assay.

Results

24hr High Concentration Neurapheresis Cycling

Figure 2. CFU reduction over 24 cycles of filtration through a 100 kDa TFF filter at a clinically high (1E7 cells/mL) organism concentration. A 5-log reduction was achieved over 24 cycles.

24hr Mid Concentration Neurapheresis Cycling

Figure 3. CFU reduction over 24 cycles of filtration through a 100 kDa TFF filter at a lower (1E5 cells/mL) organism concentration. CFU counts were reduced below the limit of detection in under 24 hours.

TFF Cryptococcal Antigen Filtration

Figure 4. Cryptococcal Antigen (CrAg) was quantified before and after passage through 100 kDa (blue) and 5kDa (red) filter units. Samples were serial diluted and tested for the lower limit of detection with a CrAg LFA assay. Both 100kDa and 5kDa achieved antigen reduction ([initial titer]-[final titer]; [1:100000]-[1:10000] and [1:100000]-[1:100], respectively).

Cytokine/Chemokine Neurapheresis Filtration

Figure 5. Infected human CSF was filtered using the Neurapheresis system and relevant cytokines were quantified. A reduction in cytokine concentration (IL-1ra, IL-6, TNF, CRP, and CXCL10) was achieved. 100kDa reduced all cytokines except IL-1ra by >95% baseline, and 5kDa reduced >95% of all cytokines quantified.

Conclusions

• Both tangential flow filter sizes cleared yeasts rapidly and efficiently.
• Over 24 cycles, we consistently observed a 5-log drop in CFUs (>99%), reducing CFU counts below the limit of detection.
• 100kDa and 5kDa achieved a substantial antigen reduction (using CrAg LFA [initial titer]-[final titer]; [1:100000]-[1:10000] and [1:100000]-[1:100], respectively).
• A similar reduction in cytokine levels (IL-1ra, IL-6, TNF, CRP, and CXCL10) in infected human CSF was also achieved (100kDa reduced all cytokines except IL-1ra by >95% baseline, and 5kDa removed >95% of all cytokines quantified).

Neurapheresis™ Therapy is capable of substantially reducing CSF CFU burden in CM. Continuous filtration for multiple cycles shows promise for rapid CSF ‘cleaning’, and future iterations may include adjunctive infusions with drug therapies to rapidly and completely eliminate yeasts. Substantial and rapid reduction of cryptococcal antigen and key inflammatory cytokines also has significant potential for controlling the neuro-inflammatory storm that accompanies CM.

Future Directions

• Minnetronix is working to translate this research to humans. We are in development of a Neurapheresis system tailored to CM (Figure 6) for future clinical evaluations.
• A first-in-human clinical trial is currently underway to evaluate the safety of the catheter-based extracorporeal system in a different patient population.

References and Acknowledgements


Acknowledgments: Research was made possible through funding from the NIH NIAID (1R41AI120354-01 & 2R42AI120304-02)

Contact Information:
Duke University: Nandan P. Lad – Nandan.Lad@duke.edu
Minnetronix: Aaron R. McCabe – amccabe@minnetronix.com

Neurapheresis is a trademark of Minnetronix, Inc.