Feasibility of Neurapheresis™ Therapy for Multidrug Resistant Gram-negative Bacterial Meningitis

Background
The World Health Organization has identified Pseudomonas, Acinetobacter and Klebsiella (PAK) as three multidrug resistant (MDR) gram-negative pathogens that pose a threat to human health. The greatest threat lies in hospitals, nursing homes, and patients with devices such as intravenous catheters and ventilators. Gram-negative bacterial meningitis (GBM) manifests when these bacteria invade the central nervous system. Due to increasing antibiotic resistance and the high mortality associated with MDR GBM, we have tested a closed-loop, extracorporeal cerebrospinal fluid (CSF) filtration system (Neurapheresis™ Therapy) for its applicability in this context. Here we demonstrate feasibility of Neurapheresis Therapy for MDR GBM and characterize system parameters for bacterial, endotoxin, and cytokine clearance.

Methods
- Bacterial cultures grown separately, diluted to 1x10^7 cells/mL in 150 mL Luria-Miller broth or artificial CSF
- Solution passed through tangential flow or dead-end filters in a single-pass or closed loop paradigm (Fig. 1)
- Sampling: immediately post filter in single pass experiments, after each cycle in closed loop experiments
- Bacterial load quantified via CFU counts, endotoxin via Limulus Amebocyte Lysate (LAL) assay, cytokines via Luminex assay

Results
- Complete removal of bacteria with filters 0.45µm or smaller (Fig. 2)
- >99% reduction of endotoxin with 5kDa filter, >95% with 100kDa filter, single-pass (Fig. 3)
- 5kDa filters reduced cytokine load 2 log (>99%) (Fig. 4)
- 1-2 Log CFU (90-99%) reduction of all bacteria over 4 filtration cycles (Fig. 5)

Conclusions
Neurapheresis shows potential to be an efficient multi-modal tool for controlling and treating MDR GBM in this in vitro model. Extending closed loop filtration over time demonstrates capability for rapid sterilization of the CSF. Future studies will include in vivo experiments to assist in the development of a human Neurapheresis system tailored to MDR GBM removal. Future iterations may include adjunctive intrathecal drug delivery to further accelerate elimination of bacteria. Reduction of bacteria, endotoxin and cytokines by Neurapheresis may have significant implications for controlling the damaging neuro-inflammatory response during MDR GBM.

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