Rabbit Model of TB Meningitis in Children

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Disclosures

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Background: CNS Tuberculosis

• WHO 2016: 1 million new childhood cases
• Central Nervous System (CNS) Tuberculosis (TB) is the most severe form of extrapulmonary TB
  – TB meningitis
  – Tuberculomas
• Disproportionately affects young children!
• Difficult to diagnose
Background: CNS Tuberculosis

• Fatal without treatment
• Despite treatment
  – Mortality is high (13-50%)
  – Morbidity is high in survivors
• Poor neurodevelopmental outcomes unique to children
  – Hydrocephalus
  – Infarcts

Background: Treatment

• Long duration
• Poor CNS penetration
  – Ethambutol, Rifampin
• No current tools for therapeutic monitoring
• Ideal target for non-invasive imaging modalities
• New additions:
  – High-dose Rifampin
  – Fluoroquinolones

Ruslami 2013, Heernskerk 2016, Thwaites 2011
Background: Microglia

- Major immune cells of brain
- Infection causes microglial activation
- Activated microglia/macrophages highly express TSPO
- Important for normal development
  - Neurodevelopmental apoptosis
  - Neurogenesis
  - Synaptogenesis

Objective

• To establish a pediatric CNS TB animal model

• To investigate microglia’s unique role in CNS TB infection in the developing brain
In-vivo Pediatric Rabbit Model

New Zealand White Rabbits
In Vivo Subarachnoid Injection of
*Mycobacterium tuberculosis* H37Rv

- **Day 1**: Birth
- **Day 4-8**: Postnatal
- **Day 14**: Gross Pathology and Immunohistochemistry
- **Day 21**: Bacillary Burden
- **Day 28**: Imaging with Biocontainment System
- **Day 35**: Neurobehavioral Testing
Bio-Safety Level 3 Facility
Exudative Meningitis and Tuberculoma Formation After Subarachnoid Infection

Infected

Control

14 Days Post-Infection

Infected

21 Days Post-Infection

Exudate

21 Days Post-Infection

Tucker et al, DMM 2016
Exudative Meningitis and Perivascular Infiltrate

H&E Staining

Tucker et al, DMM 2016
Tuberculoma with Central Necrosis & Cellular Rim

H&E Staining

Tucker et al, DMM 2016
Activated Microglia Surrounding Tuberculoma Formation

- Large Cell Bodies
- Short, Thick Processes
- Activated Microglia
- Tuberculoma

Iba-1 Microglia Stain
DAPI Nuclear Stain
40X
Subarachnoid Infection Causes Microglia Activation

**Microglia Iba-1 Quantification**

- Activated Microglia/Total Microglia
- Infected
- Uninfected

- Small Cell Bodies
- Large Cell Bodies
- Long Processes
- Short Processes
Objective

• To use noninvasive imaging modalities to:
  – Demonstrate tuberculosis-associated neuroinflammation

  AND

  – Elucidate pharmacokinetic parameters
Non-Invasive Neuroinflammation Imaging

• Tool to monitor current or novel treatments
• Radioiodinated DPA-713
$^{124}$I-DPA-713 PET/CT Imaging of Neuroinflammation

- 2nd Generation synthetic ligand of TSPO
- Highly expressed on activated microglia & macrophages
- Imaged 1 & 24 hours post-injection

Biocontainment for Bio-safely Level 3
3-D $^{124}$I-DPA-713 PET/CT

*M. tuberculosis* Infected

Uninfected Control

24 hr

Tucker et al, DMM 2016
Localization of $^{124}$I-DPA-713 Correlates with CNS TB Lesion on Gross Pathology

3 Weeks Post-Subarachnoid *M. tuberculosis* Infection in Rabbit Kits

Tucker et al, DMM 2016
$^{124}$I-DPA-713 Accumulation in CNS TB Lesion

- Infected N=2 for 1 & 24 hours
- Infected N=1 for 48 hours
- Uninfected N=1 for all time points

2 ROIs for each animal

Tucker et al, DMM 2016
Conclusions

• Established the 1st pediatric CNS TB animal model
  – Microglia activation
  – Neurologic abnormalities
  – Exudative meningitis and brain tuberculoma formation on gross pathology
  – Radioiodinated DPA-713 accumulates in tuberculomas & correlates with neuroinflammation on gross pathology & histology
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Rabbits