Molecular Imaging for Infections

Alvaro A. Ordonez, M.D.

Tuberculosis Meningitis Workshop – NIH - May 22, 2017
Questions for TB Meningitis

1. Is there *M. tuberculosis* in the brain?

2. What is the extent of that infection?

3. Is the treatment working?
Problem - Diagnosis of Bacteria

Fundamental diagnostic: 1884
Need to isolate the bug

Fundamental diagnostic: 2017
Need to isolate the bug
A T2-weighted, fluid-attenuated, inverse-recovery MRI image taken 5 days later showed several infarcts (arrows) in the basal ganglia.

Solution - Take a Picture
Imaging TB Meningitis

• Anatomical Imaging (CT, MRI)
  – CT useful to detect hydrocephalus and vascular complications
  – MRI is more sensitive than CT in determining the extent of meningeal and parenchymal involvement.
  – MR angiography – vascular disease
  – MR spectroscopy can be used to characterize tuberculomas and differentiate them from neoplasms
  – Problem: lack of specificity and delay in tissue changes

Imaging TB Meningitis

• Imaging Inflammation
  – $^{18}$F-FDG PET
    • Possible detection of earlier findings
    • Host dependent
    • Very sensible
    • Not specific

MRI

$^{18}$F-FDG PET

Prospective imaged 35 adults with MDR-TB, on second-line TB treatment, using $^{18}$F-FDG PET and CT at 2 and 6 months after starting treatment.

Imaging assessed by radiologists or automated analyses.

$^{18}$F-FDG PET at 2 months and automated CT at 6 months were more sensitive than sputum smear or solid culture conversion at 2 months, these differences were not statistically significant, possibly because of the small sample size in our study.

Automated methods were more reliable than radiologists.
Imaging TB-associated inflammation with iodo-DPA-713

Iodo-DPA-713 is a ligand for translocator protein (TSPO)
Up-regulated in inflamed microglia and macrophages
TB lesions full of activated macrophages

TSPO expression in macrophages within TB lesions

Foss et al. Journal of Infectious Diseases. 2013
125I-DPA-713 vs 18F-FDG: Imaging TB-inflammation to monitor treatments

Pulmonary 125I-DPA-713 SPECT, but not 18F-FDG PET, correctly identified the bactericidal activities of the TB treatments as early as 4 weeks after starting treatment (P < 0.03)

Iodo-DPA-713 bound activated (CD68+) antigen presenting cells and imaging correlated with tissue TNF-α (Spearman’s ρ = 0.94; P < 0.01)

Significant correlation was found between an increase in 125I-DPA-713 SPECT activity (but not with 18F-FDG PET) with bacterial burden at relapse (Spearman’s ρ = 0.79; P < 0.01)
Imaging TB-inflammation using $^{124}$I-DPA-713 PET in a Rabbit model of Pediatric TB meningitis
Infection

Identification

Efficacy

Search for small molecules metabolized by prokaryotic-specific pathways

Discriminate infection from non-infectious processes

Categorize the causative bacterial species

Provide information on antibiotic efficacy
Searching for Bacteria-Specific tracers

Library of small molecules $^{14}$C and $^3$H labeled compounds (961)

Literature Search

Absence of known eukaryotic accumulation or metabolism
- No -1
- Yes +1

Metabolized by prokaryote-specific pathways
- No -1
- Yes +1

Evidence for prokaryote accumulation or antimicrobial activity
- No -1
- Yes +1

In-vitro testing (10)

Possible

Ordonez and Weinstein et al. Journal of Nuclear Medicine, 2017
## Searching for Bacteria-Specific tracers

### Detect the presence of bacterial infection

### Identify the “type” of bacteria

<table>
<thead>
<tr>
<th>Name</th>
<th>S. aureus (Gram-positive)</th>
<th>E. coli (Gram-negative)</th>
<th>P. Aeruginosa</th>
<th>Mycobacteria*</th>
<th>Macrophages (J774)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arabinose [1-14C]</td>
<td>0.41 ± 0.03</td>
<td>41.61 ± 9.91</td>
<td>0.21 ± 0.02</td>
<td>0.28 ± 0.01 (Mtb)</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>Cellobiose [3H]</td>
<td>1.81 ± 0.10</td>
<td>0.80 ± 0.05</td>
<td>--</td>
<td>0.13 ± 0.02 (Ms)</td>
<td>--</td>
</tr>
<tr>
<td>D-Lyxoose [1-14C]</td>
<td>0.03 ± 0.01</td>
<td>1.86 ± 0.14</td>
<td>0.12 ± 0.04</td>
<td>0.35 ± 0.08 (Mtb)</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>D-Mannitol [1-14C]</td>
<td>68.40 ± 7.39</td>
<td>81.80 ± 1.96</td>
<td>0.69 ± 0.05</td>
<td>0.29 ± 0.13 (Mtb)</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>Methyl-α-D-glucopyranoside [methyl-14C]</td>
<td>11.01 ± 0.71</td>
<td>26.78 ± 0.59</td>
<td>--</td>
<td>0.11 ± 0.01 (Ms)</td>
<td>--</td>
</tr>
<tr>
<td>PABA [3,5-3H]</td>
<td>16.82 ± 1.03</td>
<td>18.99 ± 5.80</td>
<td>4.02 ± 1.11</td>
<td>32.93 ± 4.73 (Mtb)</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>L-Rhamnose [3H]</td>
<td>4.96 ± 0.13</td>
<td>4.73 ± 0.07</td>
<td>0.24 ± 0.04</td>
<td>3.82 ± 0.84 (Mtb)</td>
<td>0.60 ± 0.02</td>
</tr>
<tr>
<td>Shikimic acid [3-3H]</td>
<td>7.54 ± 0.01</td>
<td>1.52 ± 0.02</td>
<td>1.31 ± 0.02</td>
<td>0.17 ± 0.01 (Ms)</td>
<td>--</td>
</tr>
<tr>
<td>D-Sorbitol [14C] (18F-FDS)*</td>
<td>0.47 ± 0.09</td>
<td>72.20 ± 9.09</td>
<td>0.52 ± 0.46</td>
<td>--</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>D-Xylose [1-14C]</td>
<td>0.31 ± 0.01</td>
<td>73.94 ± 2.06</td>
<td>0.53 ± 0.08</td>
<td>0.18 ± 0.02 (Mtb)</td>
<td>0.19 ± 0.01</td>
</tr>
</tbody>
</table>

*Mycobacterium smegmatis = Ms or Mycobacterium tuberculosis = Mtb
† 2-[18F]-fluorodeoxyosorbitol (18F-FDS) used for uptake assays
Data represented as mean ± SD

Ordonez and Weinstein et al. *Journal of Nuclear Medicine*, 2017
Search for small molecules metabolized by prokaryotic-specific pathways

Infection

Identification

Efficacy

Discriminate infection from non-infectious processes

Categorize the causative bacterial species

Provide information on antibiotic efficacy
2-^{18}\text{F}-\text{PABA for Infection Diagnosis}

\begin{align*}
\text{PABA} & \quad \begin{array}{c}
\text{COOH} \\
\text{NH}_2
\end{array} \\
\text{dihydropterin methyl + PABA diphosphate} & \rightarrow \text{dihydropteroate synthase (folP)} \\
\text{Sulfamamides} & \rightarrow \text{7,8-dihydropteroate} \\
\text{Trimethoprim} & \rightarrow \text{7,8-dihydrofolate} \\
\text{dihydrofolate reductase} & \rightarrow \text{tetrahydrofolate}
\end{align*}

\begin{align*}
\text{COOH} & \quad \begin{array}{c}
\text{F} \\
\text{NH}_2
\end{array} \\
\text{2-Fluoro-PABA}
\end{align*}
2-\(^{18}\)F-PABA for Infection Diagnosis

In vitro uptake in M. tuberculosis

Collaboration with Stony Brook University

Ordonez and Weinstein et al. Journal of Nuclear Medicine, 2016
Search for small molecules metabolized by prokaryotic-specific pathways

- Discriminate infection from non-infectious processes
- Categorize the causative bacterial species
- Provide information on antibiotic efficacy

Infection

Identification

Efficacy

- $^{11}$C-Mannitol
- $^{2-18}$F-PABA
- $^{11}$C-PABA
- $^{18}$F-FDS
- $^{11}$C-RIF

Center for Infection and Inflammation Imaging Research
Johns Hopkins University School of Medicine
Sugar Shot for Bacteria

2-\(^{18}\)F-Fluorodeoxysorbitol (\(^{18}\)F-FDS)

In vitro uptake of $^{18}$F-FDS in Bacterial Pathogens and Mammalian cell lines

18F-FDS PET can differentiate infection sites from sterile inflammation.
18F-FDS uptake in clinical Multidrug-Resistant Enterobacteriaceae strains

- Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae
- Carbapenem-resistant Enterobacteriaceae (CRE)

Monitoring Antimicrobial Efficacy in Multidrug-Resistant Infections

Search for small molecules metabolized by prokaryotic-specific pathways

- Discriminate infection from non-infectious processes
- Categorize the causative bacterial species
- Provide information on antibiotic efficacy

Infection
Identification
Efficacy

- $^{11}$C-Mannitol
- $2-^{18}$F-PABA
- $^{11}$C-PABA
- $^{18}$F-FDS
- $^{11}$C-RIF
Are we using the right doses of antibiotics?

- Current doses based on blood levels and historic measures of efficacy
- Inappropriate levels in target tissues can lead to treatment failure, selection of resistant organisms, or toxicity/organ failure
- Rifampin, first line TB drug, with significant potential for shortening TB treatments
11C-Rifampin PET/CT of a 
M. tuberculosis-infected mouse 
post IV injection

Levels in brain are 14.55 ± 1.67% of blood concentrations.

Data represents Mean ± Standard Deviation (n=5)

Summary

• Noninvasive imaging can provide novel insights into disease pathogenesis, that may not be possible with conventional methods
  • Monitoring inflammation: $^{18}$F-FDG and $^{124}$I-DPA-713

• Need for pathogen-specific imaging

• Pipeline of bacteria-class specific imaging probes
  • $2^{18}$F-PABA – a folate precursor – promising agents for bacterial imaging (including M. tuberculosis)
  • $^{18}$F-FDS can rapidly and specifically detect Enterobacteriaceae

• PET can be used as a non-invasive alternative for measuring multi-compartment drug PK in situ and study drug penetration (and toxicities) at the site of infection, and into privileged sites (e.g. TB meningitis)
Sanjay Jain Lab
Ed Weinstein
Supriya Pokkali
Liz Tucker
Alvin Kalinda
Vikram Saini
Mariah Klunk
Peter DeMarco
Lauren Bambarger
Yongseok Chang
Allison Murawski

JHU
Carlton Lee
Maunank Shah
Kelly Dooley

JHU Radiology
Catherine Foss
Ronnie Mease
Martin Pomper
Bob Dannals
Steve Rowe
Akimos Jeffrey-Kwanisai
Ghedem Solomon
Mahadevappa Mahesh
Martin Lodge

Stony Brook University
Peter Tonge
Peter Smith-Jones
Hui Wang
Zhang Zhuo

Funding
NIH Director's Transformative Research Award R01-EB020539
NIH Director’s New Innovator Award DP2-OD006492
NHLBI R01-HL116316, R01-HL131829 and NIAID DAIDS supplement