Real-time and non-invasive, multi-compartment pharmacokinetics of $^{11}$C-Rifampin in *Mycobacterium tuberculosis*-infected mice using dynamic PET

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Why Rifampin?

- First-line treatment essential for shortening therapy against *M. tuberculosis*
- Dosing based on serum / plasma concentrations (confirmed by post mortem resection)
- Drug concentration within necrotic pulmonary lesions (post-mortem) lower than blood concentrations*

Radiosynthesis and Bioimaging of Rifampin

• Labeled first-line TB drugs with $^{11}$C
• Injected into healthy baboons

Liu et al. J Med Chem. 2010
Advantages of PET

• In the past only invasive, post-mortem techniques have been available

• PET imaging is noninvasive and does not require tissue resection
  • Radiolabeling allows for multi-compartment viewing of drug distribution

• Easily translatable to larger animals and humans for further study
C3HeB/FeJ Mouse Model

- C3HeB/FeJ mouse model develops granulomatous lung lesions with *M. tuberculosis* aerosol infection akin to human disease
  - Pulmonary lesions with caseous, necrotic centers and fibrotic edges with occasional cavitation

Pan *et al.* Nature 2005
Davis *et al.* PLoS ONE 2009
Davis *et al.* Antimicrob Agents Chemother. 2009
Granulomas

Collagen fibers (blue)

Reticulin fibers (red)
Methods

C3HeB/FeJ mice aerosol infected with *M. tuberculosis* (H37Rv)

11C-Rifampin synthesized on site

Biocontainment system to image multiple animals simultaneously

PET and CT scans co-registered using AMIDE (version 1.0.4)

Mice IV injected with radioprobe as dynamic PET scan began

Regions of interest (ROIs) drawn in multiple compartments to quantify drug distribution

Non-compartmental analysis using WinNonlin (version 2.1) for AUC and Cmax

Lung samples sent for matrix-assisted laser desorption ionization (MALDI) imaging

3D Representation of PET/CT depicting rapid localization to liver and metabolism

$^{11}$C-Rifampin PET/CT of *M. tuberculosis*-infected mouse post IV injection
Similar concentrations of $^{11}$C-Rifampin in blood of infected and uninfected animals

Data represents Mean ± Standard Deviation (n=5)
PET accurately predicts $^{11}$C-Rifampin concentrations in brain compared to blood

Levels in brain are $14.55 \pm 1.67\%$ of blood concentrations

Data represents Mean ± Standard Deviation (n=5)

11C-Rifampin accumulates rapidly in liver with no significant differences

Data represents Mean ± Standard Deviation (n=5)
Lower $^{11}$C-Rifampin concentrations in infected lung tissues

Data represents Mean ± Standard Deviation (n=5)
Lower $^{11}$C-Rifampin concentrations in infected lung tissues

- Transverse lung field PET/CT of an *M. tuberculosis* infected mouse
- Granulomatous tissue depicted by yellow circle
- Purple represents concentration of rifampin (highlighted by orange arrow)
Pharmacokinetic analysis of $^{11}$C-Rifampin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infected (n=5)</th>
<th>Uninfected (n=5)</th>
<th>$P$ value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>$30.54 \pm 1.90$</td>
<td>$34.18 \pm 2.50$</td>
<td>0.07</td>
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<tr>
<td>Injected dose (ng)</td>
<td>$0.07 \pm 0.02$</td>
<td>$0.07 \pm 0.02$</td>
<td>0.80</td>
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<tr>
<td>Injected dose (MBq)</td>
<td>$8.61 \pm 2.09$</td>
<td>$8.26 \pm 2.51$</td>
<td>0.80</td>
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<tr>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{max}$ (ng/ml)</td>
<td>$0.0622 \pm 0.029$</td>
<td>$0.0591 \pm 0.045$</td>
<td>0.89</td>
</tr>
<tr>
<td>AUC$_{0-90}$ (ng*h/ml)</td>
<td>$0.0080 \pm 0.002$</td>
<td>$0.0082 \pm 0.003$</td>
<td>0.90</td>
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<tr>
<td>Brain</td>
<td></td>
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<td></td>
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<tr>
<td>$C_{max}$ (ng/ml)</td>
<td>$0.0086 \pm 0.009$</td>
<td>$0.0050 \pm 0.002$</td>
<td>0.39</td>
</tr>
<tr>
<td>AUC$_{0-90}$ (ng*h/ml)</td>
<td>$0.0017 \pm 0.002$</td>
<td>$0.0011 \pm 0.001$</td>
<td>0.49</td>
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<tr>
<td>Liver</td>
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<tr>
<td>$C_{max}$ (ng/ml)</td>
<td>$0.0624 \pm 0.017$</td>
<td>$0.0676 \pm 0.016$</td>
<td>0.63</td>
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<tr>
<td>AUC$_{0-90}$ (ng*h/ml)</td>
<td>$0.0825 \pm 0.025$</td>
<td>$0.0908 \pm 0.022$</td>
<td>0.59</td>
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<tr>
<td>Lung</td>
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<td></td>
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<tr>
<td>$C_{max}$ (ng/ml)</td>
<td>$0.0221 \pm 0.010$</td>
<td>$0.0326 \pm 0.020$</td>
<td>0.33</td>
</tr>
<tr>
<td>AUC$_{0-90}$ (ng*h/ml)</td>
<td>$0.0050 \pm 0.001$</td>
<td>$0.0077 \pm 0.002$</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Data represents Mean ± Standard Deviation; * two-tailed Student’s t test
Concentration of Rifampin in mouse granulomas

MALDI imaging after IV dose of Rifampin
(10mg/kg body weight)

In collaboration with Dr. Prideaux and Dr. Dartois (PHRI)
Study Limitations

• PET scanner resolution (1mm)
• Cannot differentiate parent compound from metabolites
• Radioactive half-life of $^{11}$C radiolabel shorter than biological half-life of rifampin
Conclusions and Future Directions

1. Successful model for imaging $^{11}$C-Rifampin distribution *in vivo* in infected animals
   - Allows for simultaneous multi-compartment measurements compared to post-mortem tissue sampling

2. Lower concentrations of rifampin were noted in necrotic areas of granulomas relative to healthy lung tissue

3. Future Directions:
   - Imaging in our other animal models (rabbits, non-human primates) and humans
   - Studying biodistribution of novel TB drugs
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