Tissue Concentrations of *Pyrazinamide*

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Pyrazinamide (PZA)

First human use in 1952; remains essential drug
- Shortening of drug-susceptible treatment
- Active versus slow growing/persisting organisms
- Synergistic with many newly introduced drugs

PZA Pharmacokinetics
- Well absorbed; low protein binding; good CSF penetration
- Acidic pH important for activity
- Tissue penetration not well characterized
PZA Penetration into Lung

- **PZA\_lung/lesion \( \rightarrow 0.7-0.8^1 \)**
- **PZA\_lung/lesion \( \rightarrow 0.3-0.4^2 \)**
- **PZA\_lung/lesion \( \rightarrow 0.6-0.8^3 \)**

1. Lanoix et al. AAC 2016
2. Via et al. ACS Infect Dis. 2015
Study Question

How well does PZA penetrate into Pulmonary TB Lesions?

AIM

Measure the intra lesional concentration of PZA utilizing microdialysis
Specific AIMs

I. Determine serum and tissue pharmacokinetics of PZA among TB patients undergoing adjunctive surgery.

II. Determine radiological and pathological lesion characteristics associated with PZA tissue concentrations.

III. Investigate genotypic and phenotypic drug resistance profiles of *M. tuberculosis* isolates recovered from sputum and lung samples with DST and whole genome sequencing.
Methods I

I. Setting
- National Center for TB and Lung Diseases in Tbilisi, Georgia
- High rates of M/XDR-TB; ~40-60 surgical resections per year

II. Patients
- DR-TB cohort undergoing adjunctive surgical resection on PZA

III. Pharmacokinetics
- Serum samples at 0, 1, 4 & 8 hours & time of resection
- μD performed for intra lesional samples
- PZA concentrations done at U. of Florida (LC-MS/MS)

IV. Laboratory
- LJ Cultures: preoperative sputum & 5 tissue cultures
Methods II

V. Pathology
- After μD, lesion bisected and pH tested
- Half of dissected lesion formalin-fixed/paraffin-embedded
- Lesions evaluated for cellularity, necrosis, granulomas, and AFB (0-3 scale)

VI. Radiology
- When available, Chest CTs read by two Emory radiologists
- Characterized dominant resected lesion

VII. Whole Genome Sequencing
- DNA extracted for tissue cultures positive for M. tb.
- WGS performed using Illumina HiSeq2000; PZA mutations evaluated
Microdialysis (μD)

- Extracellular, unbound drug
- Must account for less than 100% recovery rate (calibration)
- No-net-flux method used

**µD using No-Net Flux Method**

**Variable flow rate µD pump**
(1 µcl/min)

PZA Perfusate Concentrations
5, 10, 30, 50 µg/ml

**µD catheter**
Membrane Length: 10
Shaft Length: 40, 60mm

Ex vivo resected cavitary lesion

Collection for analysis (up to 35-40 µcl)
### Study Results: Table 1 (n=10)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Result</th>
<th>Characteristic</th>
<th>Result (N=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>8 (80)</td>
<td>CrCl (ml/min)*</td>
<td>91 (52-155)</td>
</tr>
<tr>
<td>Georgian</td>
<td>7 (70)</td>
<td>Albumin (g/dl)*</td>
<td>4.2 (3.5-4.9)</td>
</tr>
<tr>
<td>Age*</td>
<td>30 (16-54)</td>
<td>Hemoglobin (g/dl)*</td>
<td>13.7 (12.4-15.5)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1 (10)</td>
<td>ALT*</td>
<td>18 (10-133)</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>1 (10)</td>
<td>PZA 1600 mg</td>
<td>8 (80)</td>
</tr>
<tr>
<td>Weight (kg)*</td>
<td>53 (48-71)</td>
<td>PZA (mg/kg)</td>
<td>24.7 (22.5-33.3)</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>19.5 (15-22)</td>
<td>PZA Days</td>
<td>363 (120-504)</td>
</tr>
<tr>
<td>New TB case</td>
<td>7 (70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lobectomy</td>
<td>5 (50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDR/XDR</td>
<td>6(60)/2(20)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Median value (range)
PZA Concentration Time Graph (n=10)
## Non-Compartmental Analysis*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All (n=10)</th>
<th>Expected Values^</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_e$ (h$^{-1}$)</td>
<td>0.06 (.04-.13)</td>
<td></td>
</tr>
<tr>
<td>$T_{1/2}$ (h)</td>
<td>11.7 (5.3-17.6)</td>
<td>8-11</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>2 (1.7-4)</td>
<td>1-2</td>
</tr>
<tr>
<td>$C_{max}$ (μg/ml)</td>
<td>37.8 (27.1-54.7)</td>
<td>20-60</td>
</tr>
<tr>
<td>$AUC_{last}$ *(h•μg/ml)</td>
<td>247 (70-353)</td>
<td></td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ *(h•μg/ml)</td>
<td>828 (209-1140)</td>
<td></td>
</tr>
<tr>
<td>CL/F (L/h)^^</td>
<td>1.9 (1.4-7.7)</td>
<td></td>
</tr>
<tr>
<td>V/F (L)^^</td>
<td>36.4 (29-59)</td>
<td></td>
</tr>
</tbody>
</table>

*Median values (range)

## PZA Serum & Tissue Concentrations

<table>
<thead>
<tr>
<th>ID</th>
<th>PZA $C_{\text{serum}}$</th>
<th>PZA $C_{\text{tissue}}$</th>
<th>PZA $C_{\text{tissue}}/C_{\text{serum}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&amp;</td>
<td>41.04</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>28.03</td>
<td>25.06</td>
<td>0.89</td>
</tr>
<tr>
<td>3</td>
<td>25.67</td>
<td>13.95</td>
<td>0.54</td>
</tr>
<tr>
<td>4</td>
<td>28.91</td>
<td>19.78</td>
<td>0.68</td>
</tr>
<tr>
<td>5</td>
<td>25.44</td>
<td>19.29</td>
<td>0.76</td>
</tr>
<tr>
<td>6</td>
<td>44.71</td>
<td>40.17</td>
<td>0.90</td>
</tr>
<tr>
<td>7</td>
<td>34.13</td>
<td>21.98</td>
<td>0.64</td>
</tr>
<tr>
<td>8</td>
<td>27.72</td>
<td>25.76</td>
<td>0.93</td>
</tr>
<tr>
<td>9</td>
<td>26.95</td>
<td>20.96</td>
<td>0.78</td>
</tr>
<tr>
<td>10</td>
<td>23.00</td>
<td>17.75</td>
<td>0.77</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td>27.87</td>
<td>20.96 (13.95-40.17)</td>
<td>0.77 (0.54-0.93)</td>
</tr>
</tbody>
</table>

^ Free serum concentration $\rightarrow$ PZA concentration x 0.85

& No cavitary concentration was available for subject 2 due to low dialysate volume
Correlations

A

Serum Concentration (µg/ml) vs. Dosage (mg/kg)

R* = 0.71, P = 0.02

B

Serum Concentration (µg/ml) vs. Tissue Concentrations (µg/ml)

R* = 0.88, P < 0.01

* Pearson Correlation Coefficient
Chest CT Findings (n=8)

PZA tissue concentrations similar in Cavitary & Mass Lesions (22.1 vs. 24.5 μg/ml) & (0.78 and 0.73)
Pathological Examination (n=9)

Negative Correlation between

\[ PZ_A_{tissue} \text{ and Necrosis } \rightarrow R=-0.66, P=0.04 \]
\[ PZ_A_{tissue} \text{ and AFB grade } \rightarrow R=-0.75, P=0.01 \]
Culture & pH Results

- Two patients with (+) resected tissue culture
- Median pH 5.5; 3 with > median pH (7.0, 7.2, 7.2)

**Patient 1 (XDR)**
- $C_{\text{max}} = 41.04 \, \mu g/ml$
- Pre Op Smear (-)/Cx(+)
- pH = 7.2
- 2 sputum & 5 tissue *M. tb* isolates

**Patient 2 (pre XDR)**
- $C_{\text{tissue}} = 25.06 \, \mu g/ml$
- Pre Op Smear/Cx (-)
- pH = 7.2
- 1 sputum & 3 tissue *M. tb* isolates

WGS: Same Beijing strain and DST patterns for all intra patient *M. tb* isolates; no *pncA* or *rpsA* mutations
Conclusions

Limitations

• POA concentrations not measured
• pH measured ~3 hours after resection; in future will compare immediate probe measurements to pH test strips

Summary

• Good penetration of PZA into pulmonary TB lesions (0.54-0.93)
• PZA $C_{serum}$ correlates with PZA $C_{issue}$
• High grade necrosis & AFBs assoc. with lower PZA tissue concentrations
• Most lesions with pH ≤ 5.5; two patients with positive tissue cultures had high pH (1st human lung pH measurements > 50 yrs)*

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