PZA Mechanism of Action: Insights from Transcriptional Profiling

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Suggested mechanisms of action for PZA

- Disruption of membrane function
- Acidification of cytoplasm
- Inhibition of FAS I
- Inhibition of trans-translation
- Interference with NAD metabolism

Disruption of membrane function

- Translational inhibitors: Tetracycline, Streptomycin, Amikacin, Rammuthomycin, Capromycin
- Amidine: PZA, NAM, RZA
- CPZ(2), TRZ(2), TRC
- KCN, KCN + GSNO, DCDD
- CPZ(1), TRZ(1), CPZ + GSNO
- Clofazimine, Clofazimine + GSNO, azoles
- NaN₃
- Menaquinone, menadione + GSNO

1. Pyridoxacetone, desferoxamine, dipyradyl
2. Pyridoxacetone, desferoxamine, dipyradyl

- Ethanol, diamine analogs
- Ampicillin, cephalaxin
- Ceftriaxone
- Theobromycin
- Folinic acid, tetrahydrofolic acid

- GSNO
- C-source: palmitate or succinate

- Extracellular acid stress
- Hypoxia (NRF-1)
- Nutrient starvation

- DNA damaging agents: fluoroquinolones
- DNA damaging agents: UV, Mms, H₂O₂

- Transcriptional inhibitors: Rifampicin and Rifapentine
Acidification of cytoplasm
Inhibition of FAS I
Inhibition of trans-translation

• What would be the impact of inhibition of trans-translation on transcriptional profiles?
• In the absence of gene expression data on this, we will leave the relevance of this potential mechanism to be weighed by others based on other data types.
Interference with NAD metabolism

- Is there a data set that will allow this comparison?
- Yes, but has substantial differences from the Boshoff et al. 2004 data set

NAD+ auxotrophy is bacteriocidal for the tubercle bacilli.

PZA treatment and NAm starvation kill with similar kinetics
Substantial intersection of PZA 16hr w/ *M. bovis* NAD starvation

Intersection includes heat shock, protease, antioxidant, aerobic, electron transport, transcription/translation, transport (mmpLs), and lipid anabolism genes
So, proximal PZA mode of action is consistent with disrupted NAD metabolism, what’s the target?

Hypothesis:
PZA activity requires sensitizing the cell - something up or down at low pH – it’s not the pH that’s important, it’s the induction / repression (may happen in vivo at higher pH)

Rationale:
Low extracellular pH enhances POA accumulation but does not lower cytoplasmic pH, could the target be repressed at low pH, making it more vulnerable?
Low pH may sensitize *M. tb* to perturbations to NAD(P) metabolism

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<thead>
<tr>
<th>pH 5.5 up</th>
<th>pH 5.5 down</th>
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<tr>
<td>protease</td>
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<td>heat shock</td>
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<td><em>ndh</em></td>
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Annotated pH-regulated genes have a preponderance of genes that code for NAD(P) requiring or synthesizing enzymes

Conclusions

- PZA mode of action is consistent with disruption of NAD metabolism
- Low pH may sensitize *M. tb* to perturbations of NAD(P) metabolism
- Overexpression of low pH downregulated essential genes that require NAD(P) may increase PZA resistance and reveal target/s
Cofactor concentrations in mc²6230 grown at normal pH (6.8) vs pH 5.5
Effect of POA and PZA on Ndh type I and Ndh type II activities

The membrane fraction was isolated from 100 ml mc²6230 culture (OD =0.8) and resuspended in 0.4 ml MES buffer (pH 5.5).
10 µl of membrane fraction was treated with POA or PZA at rt for 30 minutes in the presence of Ndh activity.
Effect of PZA on Ndh type I and Ndh type II activities

mc²6230, grown in 7H9-OADC-gly-MES-tylo (pH 6.0), was treated with PZA (0.8 mg/ml) for 3 days prior to membrane fraction isolation and measurement of Ndh type I and type II activities.
Nucleotide concentrations

Growth of mc^26230 in 7H9-OADC-MES (pH 6.0) treated or not with PZA (0.8 mg/ml)
Growth of H37Rv with PZA at pH 6.0