Anti-Tuberculosis Activity of Pyrazinamide Varies by Lesion Type in C3HeB/FeJ Mice

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Introduction

• Humans develop a wide variety of lesion types when infected with *M. tuberculosis*.

• Commonly used mouse models develop only intracellular lesions.
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- Humans develop a wide variety of lesion types when infected with *M. tuberculosis*.
- Commonly used mouse models develop only intracellular lesions.
- Like humans, C3HeB/FeJ mice develop necrotic granulomas, caseous pneumonia and, occasionally, cavities.

Davis et al, 2009
Introduction

• If caseous necrosis is a determinant of drug effect, then C3HeB/FeJ mice may be more representative of a drug’s activity in humans.

• Pyrazinamide (PZA) is an interesting drug to study in C3HeB/FeJ mice because:
  – It requires a unique environmental condition (low pH) to be active at achievable concentrations
  – It is 1 of only 2 drugs with sterilizing activity
  – It has no sterilizing activity beyond the first 2 months of treatment with the 1^{st}-line regimen
• These curious characteristics suggest **PZA acts against a specific sub-population** of persisting bacteria residing in an acidic milieu which is not as susceptible to other anti-TB drugs.

• To better understand the relationship between lesion type and PZA activity, we investigated the pharmacodynamics of PZA in C3HeB/FeJ and BALB/c mice.
Objectives

• Compare the dose-ranging activity of PZA against established Mtb infection in both strains
• Describe the pharmacokinetics of PZA in plasma, epithelial lining fluid and lung lesions of C3HeB/FeJ mice
• Measure the pH of liquefied caseum in the lung lesions of C3HeB/FeJ mice
Dose-dependent activity of PZA in BALB/c mice

![Graph showing CFU count (log10/lung) against Dose (log mg/kg) for W3 and W8 groups.](image-url)
Dose-dependent activity of PZA in some C3HeB/FeJ mice
"Dichotomous" activity of PZA in C3HeB/FeJ mice
Why does PZA exhibit no dose-response in large, caseous lesions?

- Selection of PZA-resistant mutants?
- Lack of dose-proportional PK in plasma? In epithelial lining fluid (ELF)?
- Lack of penetration into caseous lesions?
- Lack of sufficiently acidic conditions in caseum?
Resistance?

• “Breakthrough” of PZA-resistant mutants was not found in the outliers but in mice where PZA had bactericidal activity.
Dose-proportional PK in plasma?

- 30 mg/kg
- 150 mg/kg
- 300 mg/kg
- 450 mg/kg BID

PZA concentrations (µg/ml) vs. Time (hours)
Dose-proportional PK in ELF?

- 30 mg/kg
- 150 mg/kg
- 300 mg/kg
- 450 mg/kg BID
- Lower limit of quantification

PZA concentrations (µg/ml)

Time (hours)
Penetration in lesions?

Plasma concentration of Z 150mg/kg in C3HeB/FeJ mice

AUC [0-t]= 578 h.µg.ml⁻¹
Cmax = 189.7 µg/ml

AUC [0-t]= 456 h.µg.ml⁻¹
Cmax = 166 µg/ml
Penetration into lesions?

BAL concentration of Z 150mg/kg in C3HeB/FeJ mice

- Uninfected
- Infected
- Intralional
- Lower limit of quantification

PZA concentrations (µg/ml) vs Time (hours)
pH of caseum?

- 13 liquefied lesions tested with needle probe:
  - pH=7.39 ± 0.096 (range [7.19 - 7.54])
**pH of caseum?**

- 13 liquefied lesions tested with needle probe:
  - pH = 7.39 ± 0.096 (range [7.19 - 7.54])
Conclusions

- PZA has lesion-dependent activity in a C3HeB/FeJ mouse model TB
- The pH of liquefied caseum in this model is not sufficiently acidic to enable PZA activity, even at drug exposures exceeding maximal human exposures
- Z most likely exerts its unique sterilizing activity inside activated macrophages, where pH can be $\leq 5$, and not in acellular caseum, where the pH is neutral
Ongoing study

- Assess the contribution of PZA to the first-line regimen in C3HeB/FeJ mice
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