Working Group on new TB drugs

Essentiality of PZA Workshop
May 31 – June 1, 2011 at NIAID/NIH

Highlights, Conclusions, Action Items

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- **WGND convened a small workshop to explore the current state of knowledge regarding PZA mechanisms of action and biological effects**
- **Invited NIAID-supported investigators to compare results and discuss future research**

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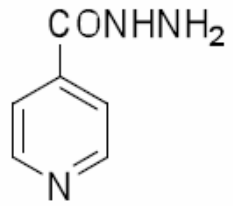
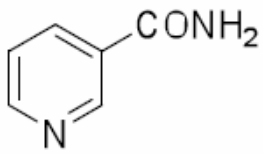
Isoniazid

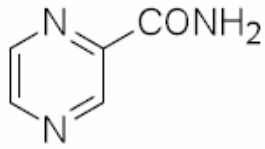


Chemical Formula: $C_6H_7N_3O$
Molecular Weight: 137.14
CLogP: -0.668

Nicotinamide



Pyrazinamide



Chemical Formula: $C_5H_5N_3O$
Molecular Weight: 123.11
CLogP: -0.67632

- Discovered in 1952 by testing limited nicotinamide analogs in mouse (Dessau, et al., Am. Rev. Tuberc., 1952, 65, 635)
- Pro-drug – requires activation by pyrazinamidase (PncA) to pyrazinoic acid
- Narrow spectrum – mycobacteria only
- Low, pH dependent activity – MIC ≥ 16 µg/ml (at pH 5.5)
- Potent *in vivo* efficacy against chronic but not acute infection in mice
- Dose-limiting toxicities – hepatotoxicity and arthralgia

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In vitro data:

- PZA analogs have greater in vitro activity against *M. tuberculosis*
- 5-Cl PZA and PZA have been shown to be competitive inhibitors of Mtb FAS I
- PZA and NADPH compete for binding to Mtb FAS I by NMR
- POA and PZA do not compete for binding to Mtb FAS I by NMR
- PZA may disrupt NAD synthesis in *M. tuberculosis*

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Experimental animal *in vivo* data:

1. **In vivo data suggest there may be mutual antagonism between INH and PZA**

2. **PZA improves the activity of virtually all new drugs in clinical development in murine models of TB**

3. **The sterilizing potential of PZA extends beyond the first 2 months of treatment in murine models**

4. **Many of the older observations with nicotinamide and PZA should be re-visited with modern technology.**

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Mode of Action of PZA:

1. **PZA as a structural analog of nicotinamide could interfere with NAD+ salvage pathway that uses nicotinamide to synthesize NAD+. Experiments were proposed to test this hypothesis.**

2. **PZA has clear-cut activity against non-replicating bacilli but no activity at all against actively replicating bacilli in the nude mouse model.**

   Accumulation of toxic products within non-replicating bacilli may be the essential mechanism by which pyrazinamide kills tubercle bacilli.

3. **RpsA, a new target for POA? A key role in *trans*-translation - a rescue process?**

4. **A new generation of PZA derivatives active against PZA-resistant *M. tuberculosis* and with a wider therapeutic window are needed and are being explored by the TB Alliance.**

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PZA, an immunomodulator? Observations:

- **PZA has activity against *Leishmania* in vivo in models of cutaneous and visceral disease**
- **PZA analogs (i.e. 5-chloro and 5-fluoro) have greater activity than the parental compound. The target for PZA in *Leishmania* is unknown, but it has been hypothesized that the drug inhibits the activity of either microsomal elongases or sirtuins in this parasite**
- **In uninfected and *M. tuberculosis*-infected J774 cells, the addition of PZA, nicotinamide, POA, or nicotinic acid did not induce a pro-inflammatory cytokine response**
- **PZA administered for 25 days by oral gavage was associated with a pro-inflammatory cytokine response in the lungs of both uninfected and *M. tuberculosis*-infected C3HeB/FeJ mice**

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What we learned:

1. **PZA's conversion to pyrazinoic acid has multiple effects in the mycobacterial cell: acidic stress, potential inhibition of fatty acid synthesis, chelation of di-cationic metals, stalling of ribosomes, inhibition of NAD synthesis.**
2. **PZA is essential for the most effective combination regimens in mice and human TB studies, and is active against a few other organisms (e.g. *Leishmania*)**
3. **PZA is bactericidal in immune competent mice but appears to be less active in mice with immune defects.**
4. **Mutations at any point in the pncA gene can cause loss of pyrazinamidase enzyme activity leading to resistance**
5. **Binding of POA to RpsA might compromise the rescue process of *trans*-translation**
6. **Timing of use beyond the initiation phase of treatment should be discussed.**

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Next steps, Next questions

1. **More research is needed on mechanism(s) of action including the potential target, the range of susceptible organisms, the role of acidic environment**

2. **Past and new analogues: Why does the addition of a nitrogen atom in the pyridine ring increase by ~ 7 times the anti-TB activity of nicotinamide? What are the similarities with respect to mechanism of action between the pyrazine and pyridine scaffolds? Is nicotinamide synergistic with rifampin?**

3. **Drug susceptibility test: Will we evolve soon from phenotypic to genotypic testing? With new technology, can PZA susceptibility become a rapid and routine "automated" test?**

4. **Timing of regimen use: why is such an unique sterilizing drug used only during the initial (bactericidal) phase and not in the continuation (sterilizing) phase of treatment for tuberculosis?**

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Outcomes from the Workshop

1. **Agreed to help convene a larger NIAID conference in the Fall 2012**

2. **Posted presentations, videos, and synopses from speakers on the working group website: http://www.newtbdrugs.org/meetings/pza-workshop.php**

3. **Contributed to the search for pyrazinamide resistant strains without mutations in PncA**

4. **Fostered collaborations with the CDC, NIAID, TB Alliance, and CPTR to advance PZA diagnostics research and development**

5. **Focused attention on a key sterilizing drug and identified important knowledge gaps in its basic mechanisms**