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New insights into the mechanism of action of pyrazinamide, implications for susceptibility testing, and future regimens ☆

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ABSTRACT

Pyrazinamide (PZA) is included in the 2016 World Health Organization multidrug-resistant tuberculosis treatment guidelines and is a key component of most ongoing clinical trials investigating novel antibiotic combinations. PZA resistance is associated with worse tuberculosis treatment outcomes. Unfortunately, for such an important drug, phenotypic susceptibility testing is extremely challenging. The exacting bacterial growth conditions required to induce susceptibility to the drug reduce the accuracy of the susceptibility assay, even in experienced laboratories, and widespread testing is not performed. This situation is unacceptable for such a valuable and important drug. A more complete understanding of the mechanism of action of PZA would be expected to lead to improvements in this situation.

Although the exact mechanism of action of PZA is not known yet, it is widely accepted that PZA is a prodrug requiring transformation to pyrazinoic acid, the active form, by the mycobacterial enzyme encoded by the *pncA* gene. Most clinical resistance indeed appears to be a result of a diverse range of mutations in this gene and sequencing of the *pncA* gene has been shown to have excellent predictive power for PZA resistance. The wider availably of *pncA* sequencing in combination with databases of the phenotypic implications of these mutations has helped make genetic testing for PZA resistance a practical proposition.

For the past decades, it has been generally accepted that an extracellular low pH is required for PZA activity but work in our laboratory [1] and others [2] has recently challenged this assumption. Alternative bacterial stresses, apart from a reduced pH of the growth media (such as reduced temperature), can also induce a PZA-susceptible phenotype. The characterization of spontaneous *in vitro*-resistant pyrazinoic acid mutants selected under neutral pH conditions suggests a key role for the pantothenate/coenzyme

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A biosynthetic pathway. This has profound implications for the mechanism of action of PZA as well as potentially the bacterial population against which PZA is active in the host.

These findings will be discussed as well as their implications for further research and the future of PZA susceptibility testing.

Conflicts of interest

The authors have no conflicts of interest to declare.

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