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"Combating tuberculosis through an in-depth understanding of a *Mycobacterium tuberculosis* tolerance mechanism and the development of novel pyrazinoic acid prodrugs"

by
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Wednesday, August 4, 2021
1:00 P.M.
https://rutgers.zoom.us/j/96752714639?pwd=TC9rR0VmZUZTZUdpb3Bra1JVQzk4Zz09
Meeting ID: 967 5271 4639
Password: 268511

ABSTRACT

The standard drug regimen to combat tuberculosis (TB) is often hindered by emergence of drug-resistant and/or incomplete clearance of drug-tolerant *Mycobacterium tuberculosis* (Mtb) populations. Drug resistance is associated with genetically encoded mutations in drug targets or drug activators that confer survival to an otherwise lethal concentration of drug. This contrasts to drug tolerance, which has traditionally been attributed to activation of two component systems, stochastic events, and phase variation all of which led to a state of reduced growth that is induced by drug or immune system pressure and can be reversed once the inducing state is removed. A better understanding of the fundamental mechanisms of drug tolerance will yield new drug targets that can be exploited to develop better compounds active against Mtb. **Part I:** Unstable frameshift mutations in a homopolymeric tract of the glycerol kinase gene (*glpK*) leads to phase variation and a reversible small colony variant (SCV) phenotype. These mutants are drug-tolerant, enriched in clinical isolates, and associated with extensively drug-resistant Mtb. A hallmark of drug-tolerant Mtb is redirection of carbon flux away from energy metabolism and into lipids. We hypothesized that deletion of *glpK* in Mtb would result in an irreversible tolerant state due to decreased glycerol-3-phosphate (G3P) flux towards energy metabolism and increased flux towards lipid anabolism. We found that deletion of *glpK* in laboratory Mtb strain H37Rv emulated the SCV and drug-tolerant phenomena observed in clinical *glpK* mutants. Analysis of the transcriptional profile revealed that the *glpK* deletion mutant was in a quiescent state characterized by an increase in the expression of dormancy regulon genes. The irreversible *glpK* mutant also had a higher neutral lipid content and was recalcitrant to acid fast staining, suggesting alterations in cell wall architecture. Transmission electron microscopy identified accumulations of outer cell wall components that were further defined using lipidomics as phthiocerol dimycoserosates. Furthermore, transposon sequencing revealed that the *glpK* mutant was more depended on genes responsible for lipid anabolism and less dependent on genes involved in fluxing G3P into central carbon metabolism. Together, these data implicate the functional status of *glpK* in the transition of Mtb into a dormant state. **Part II:** Introduction of pyrazinamide (PZA) to the first-line anti-TB treatment regimen resulted in shortening of therapy from twelve to six months and consequently played a critical role in curbing drug resistance that develops over long course therapy. PZA is a prodrug activated by a nonessential amidase, PncA, to release its active moiety pyrazinoic acid (POA). Resistance to PZA can develop and often results in treatment failure. Thus, drugs with treatment shortening potential like PZA are urgently needed. Here, we used a whole-cell drug screening approach to identify anti-tuberculars with unconventional mechanisms of action or activation that could be further developed into compounds effective at killing Mtb resistant to PZA. We discovered an amide containing prodrug, DG160, that was activated by an amidase, Rv2888c (AmiC). This amidase could metabolize a variety of amide containing compounds including a novel POA-DG160 prodrug, JSF-4302, developed in this study. We found that AmiC activation of JSF-4302 led to generation of POA in a PZA-resistant clinical isolate, thereby successfully delivering the active component of PZA while bypassing the need for activation by PncA. This work provides a framework for a new approach to drug development and prodrug activation in Mtb.