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"Exploiting vulnerabilities in *Mycobacterium tuberculosis* respiration to identify new drugs and drug targets"

By
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2:00 P.M.
ICPH - 1st Floor Auditorium

ABSTRACT

Tuberculosis (TB) is one of the leading causes of death worldwide due to a single infectious agent. It is caused by the acid-fast bacteria *Mycobacterium tuberculosis* (*Mtb*). Existing treatment for drug-susceptible TB infections requires the administration of a cocktail of drugs for a minimum of six months. This creates many opportunities for noncompliance and drug toxicity, which can lead to treatment failure, relapse, and the emergence of drug resistance. New drugs to treat TB are urgently required. It is well known that *Mtb* occurs in a number of metabolic and growth states ranging from metabolically active replicating *Mtb* in well aerated cavities to slowly replicating or even non growing persitant *Mtb* in hypoxic and nutrient limited granulomas. However, respiration is critical for survival in all of these states. We hypothesize that drugs that target bacterial respiration will kill both actively growing and persistent bacteria. These new drugs should aid in reducing treatment duration and should also be effective against clinical drug-resistant cases of TB. To address this, we developed two novel whole-cell respiratory pathway specific screens to identify compounds that killed *Mtb* by inhibiting its respiration. This led to the identification of two small molecules DG70 and DG77. Various biochemical tests confirmed the effect of these compounds on bacterial respiration. Whole-genome sequencing of DG70-resistant colonies identified mutations in *menG* (*Rv0558*), which is responsible for the final step in menaquinone biosynthesis and essential for respiration. Genetic, radiolabeling, and high-resolution mass spectrometry studies confirmed that DG70 inhibited the final step in menaquinone biosynthesis. DG70 and DG77 showed potent bactericidal activity in both actively replicating cultures and in well established *in vitro* model of *Mtb* persistence. We noted that DG77 had a resistance frequency of less than 10^{-9} CFU/ml indicating a high barrier to resistance compared to all other known TB drugs. We then performed intra-bacterial drug metabolism and transposon sequencing studies on DG77-treated *Mtb* to further investigate the cause of this resistance barrier. We determined that that DG77 is a prodrug which is hydrolyzed into two active pharmacophores, 2-nathahydrazide (NAP) and 5-nitro-2-furoic acid (5NF). 5NF appeared to require additional activation which could be accomplished through the action of at least two-three different F_{420} dependant nitroreductases, as well as additional nitroreductases that were not dependant on F_{420}. NAP activity was increased in *Mtb* that had acquired resistance to 5NF. Kill curve kinetic studies performed with DG77 or its two metabolites showed that NAP and a methyl ester of 5NF (M5NF) were synergistic with each other, leading to rapid sterilization of active and persistent *Mtb* cultures. Our overall results demonstrate that compounds which target respiration in *Mtb* can provide highly effective cidal activity against both growing and persistent cultures. Our detailed study of DG77 demonstrates drug-features that provide a very high barrier to resistance