

## Targeting Mycothiol Biosynthesis and Mycothiol-Dependent Detoxification for the Treatment of Tuberculosis

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Tuberculosis (TB) is an infectious disease that dates back to ancient and historical times [1]. *Mycobacterium tuberculosis* is the primary organism responsible for TB, the number two single agent infectious disease killer worldwide behind HIV [2]. In spite of the fact that there are currently effective drugs for the treatment of TB available, this disease continues to be a global problem. In 2011, there were an estimated 8.7 million new cases of TB and 1.4 million deaths attributed to TB worldwide [3]. Treatment of TB is difficult, requires multidrug therapy for extended periods of time (6-9 months), and is often unsuccessful [4-6]. The difficulty in TB treatment is attributed to the slow growth rate and resilience of the *M. tuberculosis* organism in adapting to changes in oxygen and nitric oxide levels, pH, metal ion concentrations, and available nutrients that allow for mycobacterial survival in host macrophages [7-9], as well as the high degree of drug resistance associated with *M. tuberculosis*. Resistance to existing antibiotics, including multidrug resistant (MDR)- and extensively drug resistant (XDR)-TB, presents a significant challenge in TB treatment as it further limits the number of effective treatment options [5,6,10-12]. There were 310,000 cases of MDR-TB reported in 2011 (estimated ~4% new cases and 20% previously treated cases are MDR-TB), of which an estimated 9% are thought to be XDR-TB [3]. Although not widely accepted, the existence of totally drug resistant (TDR)-TB has been suggested [5,13,14]. The high number of reported MDR- and XDR-TB cases highlights the need for new drug agents, including those that act via new targets to circumvent existing mechanisms of drug resistance.

Infection with *M. tuberculosis* can cause two types of diseases – symptomatic active TB infection (replicating bacteria) or asymptomatic latent TB infection (LTBI, non-replicating bacteria). Current treatment regimens for active TB are comprised of two phases, an initial phase and a continuous phase [4,5]. The initial phase of therapy consists of combination treatment with four first-line agents, most commonly isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB), for two months. This is followed by a continuous phase of therapy with two first-line agents for an additional 4-7 months to eradicate any non-replicating persistent *M. tuberculosis* present. If one or more first-line agents cannot be used for any reason (e.g., drug allergies, drug-drug interactions, drug-disease interactions, poor drug tolerability, drug resistance), one or more second- (e.g., streptomycin, SM, ethionamide, ETA) or third-line agents are included in the treatment regimen [4-6]. While this approach to treatment is effective, it has a number of disadvantages associated with it that often hinder completion of the drug regimen and prevent complete eradication of the disease. This includes the number of drugs required, length of treatment, and poor drug tolerance (e.g., hepatotoxicity). Treatment of TB is especially problematic in patients with HIV on antiretroviral therapy (ART) due to drug-drug interactions [5,15,16]. RIF is a potent inducer of cytochrome 450 3A (CYP3A), and co-administration of RIF with certain ART drugs (e.g., protease inhibitors, non-nucleoside reverse transcriptase inhibitors) results in decreased concentrations of ART drugs that are metabolized by CYP3A. This is a significant problem as TB is the number one cause of death and most common presenting illness in people with HIV [3]. Consequently, there is tremendous need for new TB drugs with enhanced efficacy and improved drug tolerance

that can shorten the length of treatment regimen and improve patient compliance to ensure eradication of the disease.

LTBI presents an additional therapeutic challenge as the *M. tuberculosis* present is often in a non-replicating and persistent state [5,15,16]. Several changes in metabolism take place when *M. tuberculosis* transitions from the active to latent form, including redirection of the carbon flux from the TCA cycle to triacylglycerol, upregulation of the  $\beta$ -oxidation pathway, and changes in respiration (i.e., electron transport chain pathway, terminal electron acceptor) [9,17]. These metabolic changes can result in unresponsiveness to existing drugs, further limiting treatment options. LTBI is most commonly treated with a single agent (i.e., INH, RIF) for 9-36 months [5,15-17]. Due to the toxicities, drug-drug interactions, and drug-disease interactions associated with these drugs, LTBI is only treated in at risk populations, such as individuals with HIV, end-stage renal failure, or patients on immunosuppressive/immunomodulatory therapy. LTBI is especially of concern in HIV patients. It is estimated that at least one-third of the 34 million people infected with HIV are also infected with TB, and these individuals with LTBI are 21-34 times more likely to develop active TB compared to the persons without HIV [3]. Treatment of LTBI is significantly hampered by poor drug tolerance (e.g., hepatotoxicity), length of treatment, and RIF-ART drug interactions which impact patient compliance and hinder disease eradication. Consequently, there is a need for new drugs with increased efficacy, shortened drug regimens, and improved tolerability. The recent shortage of INH in the United States (2012-2013) had a significant impact on LTBI treatment, including delays in LTBI treatment and changes to alternative LTBI treatment regimens [18]. This further highlights the need for additional drugs that are effective in treating LTBI, including those that act on new drug targets.

One potential source of new drug targets for the treatment of TB is mycothiol (MSH). MSH is the biological thiol used by mycobacteria to maintain an intracellular reducing environment and to protect against cellular damage from oxidative species and xenobiotics, analogous to the functions provided by glutathione (GSH) in other species [9,19-21]. Since MSH is unique to mycobacteria, it is an attractive drug target for the treatment of TB. MSH is synthesized from inositol-1-phosphate and UDP-N-acetyl-glucosamine using five proteins (MshA, MshA2, MshB, MshC, MshD) [19-21]. Although there is some controversy as to whether or not specific genes involved in MSH biosynthesis are essential

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in *M. tuberculosis*, it is generally accepted that the critical functions provided by MSH enable mycobacteria to tolerate and withstand the oxidative, nitrosative, and other stressed conditions required for mycobacterial survival in the host. Importantly, current data indicate that MSH plays an important role in non-replicating persistent bacteria suggesting that MSH biosynthesis may be a source of potential targets for the treatment LTBI [9,22,23]. Mycobacteria also contain a number of MSH-dependent enzymes that serve important roles in maintaining a reduced environment and in the detoxification of xenobiotics, similar to what is observed with GSH. These include a mycothiol reductase (Mtr) that maintains MSH in the reduced form, an oxidoreductase (Mrx1) that reduces S-mycothiolated mixed disulfides, mycothiol-S-transferase (MST) that catalyzes conjugation reactions with MSH, and a metalloamidase (MCA) that hydrolyzes MS-conjugates [19]. In light of the important functions that MSH provides and the fact that these pathways are absent in humans, the MSH biosynthetic and MSH-dependent detoxification pathways may be sources of targets for the treatment of TB, including LTBI.

Importantly, MSH may play a role in modulating the sensitivity of mycobacteria to existing TB antibiotics [19]. Mycobacteria lacking MSH exhibit enhanced (e.g., RIF, SM) and decreased (e.g., INH, ETA) sensitivity to existing TB drugs. These findings suggest that MSH may be required for the activation of some TB drugs (i.e., INH, ETA) and contribute to the resistance observed for other TB drugs (i.e., RIF, SM). Consequently, multidrug regimens would need to be managed to avoid concomitant use of drugs that decrease MSH levels with drugs that require MSH for activation (e.g., INH and ETA), while taking advantage of the potential synergy between drugs that decrease MSH levels and drugs undergo MSH-dependent detoxification (e.g., RIF and SM) to enhance effectiveness of existing antibiotics.

Significant efforts have been made over the last several years to identify and characterize the enzymes involved in MSH biosynthesis and MSH-dependent detoxification so that these enzymes can be targeted for the development of inhibitors as potential antibiotics for the treatment of TB. Both MSH biosynthesis inhibitors (MBIs) and MSH-dependent detoxification inhibitors (MDIs) have been reported and were recently reviewed [19]. Importantly, the best inhibitors have demonstrated the ability to inhibit the growth of *M. tuberculosis* under aerobic and anaerobic conditions, and to kill non-replicating *M. tuberculosis*, validating these pathways as sources of drug targets. Furthermore, the ability of MshC inhibitors to target both replicating and non-replicating *M. tuberculosis* suggests that targeting MSH biosynthesis may be an effective strategy for the development of new treatment options for LTBI. The development of a new class of drugs that can effectively target non-replicating persistent *M. tuberculosis* would be an important and much needed advancement for the treatment LTBI in HIV patients on antiretroviral therapy.

The potential usefulness of MBIs and MDIs in the treatment of active TB and LTBI can be summarized as follows. Although MBIs have the potential to work synergistically with the first-line TB agent RIF, since treatment of active TB requires a multidrug regimen that most commonly consists of INH, RIF, EMB and PZA, MBIs are not likely to be a viable option for treatment of active TB since they would decrease the effectiveness of INH. However, MDIs may be suitable for use in treatment of active TB as these inhibitors have the potential to circumvent MSH-dependent resistance associated with drugs such as RIF and SM, while not affecting drugs that require MSH for activation (i.e., INH, ETA). The viability of this approach should be further investigated. In terms of LTBI, MBIs may be a viable treatment strategy

alone or in combination with RIF. Additional studies need to be carried out to further explore this possibility, as well as the suitability of MDIs in treatment of LTBI.

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