Role of Malate Synthase and other Enzyme Inhibitors in *Mycobacterium Tuberculosis*: A Mini Review

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Running Title: Malate Synthase and other Enzyme Inhibitors in *Mtb*

Abstract- The emergence of multidrug-resistant strains of *Mycobacterium tuberculosis* (*Mtb*) has intensified efforts to discover novel drugs for tuberculosis (TB) treatment. Targeting the persistent state of *Mtb*, a condition in which *Mtb* is resistant to conventional drug therapies, is of particular interest. Persistent bacteria rely on metabolic pathways that are distinct from active infection *Mtb* because persistent *Mtb* are forced to survive in a low nutrient environment and various enzyme pathways. We are studying malate synthase (MS), one of the enzymes in the glyoxylate shunt and their potential inhibitors. Inhibitors against MS, Proteasome, Protease, Phospho-N-acetylmuramyl-pentapeptide translocase, Topoisomerase, ATP Synthase have been characterized for further development into potential novel antitubercular drugs.

Keywords- Malate Synthase, Proteasome, Protease, Topoisomerase, *M. Tuberculosis*.

I. INTRODUCTION

Tuberculosis (TB) is an infectious disease, leading cause of death worldwide, primarily in developing countries. Over one third of the world’s population is infected with TB with approximately 8 million new cases of infection every year. TB incidence is also on the rise because of the correspondingly high HIV infection rates, both diseases progress at faster rates in co-infected individuals.

*Mycobacterium tuberculosis* (*Mtb*) is the causative agent of tuberculosis. The primary mode of transmission of *Mtb* is through the air in an aerosolized form, most commonly via a cough or sneeze. In this early stage of infection, *Mtb* is able to replicate within non-activated macrophages. However, the body subsequently mounts a cell-mediated immune response to the growing *Mtb*, which includes the activation of macrophages with interferon-γ. The cell-mediated immune response is sufficient 90-95% of the time in controlling the *Mtb* infection, but not completely eradicating the mycobacteria from the host. In fact, the remaining mycobacteria will enter into the non-replicating persistence phase or the so called latent stage of the infection. *Mtb* can reside in alveolar macrophages, avoiding the host immune response for an indefinite period of time. Activation of such latent mycobacteria can occur anytime later in life, especially when the host has become immune-compromised [1-5].

Anti-TB drug therapy has existed since the late 1940s, but the long time course of the drugs required for treatment (6-12 months) often causes low patient compliance in completing prescribed regimen. Another problem is the fact that *Mtb* can survive for extended periods of time in its non-replicative or persistent state. While in this state, *Mtb* is resistant to conventional forms of chemotherapy. Patient noncompliance coupled with the ability of *Mtb* to enter the persistence state has contributed to the emergence of multidrug-resistant (MDR) and extensively drug resistant (XDR) strains of *Mtb*. The ineffectiveness of current anti-TB drugs against persistent, MDR-TB, and XDR-TB has greatly energized the search for novel anti-TB drugs, including those that target the persistence state. In order to target the latent stage of the infection, studies have been performed to better understand the extreme intracellular conditions within host macrophages under which dormant *Mtb* reside. Some of the identified conditions include hypoxia, iron deficiency, low pH, and a fatty acid rich carbon pool all contribute to the altered phenotype that *Mtb* displays in the dormant state. Using microarray technology, genes that are up regulated in response to nutrient starvation and latency-like conditions have been identified. The complete genomic sequence coupled with microarray technology has been critical for the identification of metabolic pathways that are required to
support the persistent state in Mtb [6-12]. One of the changes that occur once Mtb enters the persistent state is an activation of the glyoxylate shunt within the tricarboxylic acid (TCA) cycle. This activation facilitates a metabolic shift to acetyl CoA as the primary carbon source, which is the product of the \( \text{I-oxidation of fatty acids.} \)

The glyoxylate shunt consists of two enzymes, isocitrate lyase (ICL) and malate synthase (MS), which act as a bypass to the two CO\(_2\) producing steps in the TCA cycle, thus conserving carbon (which can be used for gluconeogenesis) and replenishing TCA cycle intermediates in an anaplerotic fashion. The first enzyme in the glyoxylate shunt, ICL, catalyzes the cleavage of isocitrate to succinate and glyoxylate. In the second step, MS condenses glyoxylate and acetyl CoA to produce malate. Since neither ICL nor MS are present in mammals, they have become attractive targets for novel anti-TB drug design. In Mtb, the genes encoding both ICL and MS are upregulated in response to macrophage phagocytosis. It has been reported that an Mtb ICL knockout strain was able to establish an acute, but not a persistent infection in mice. Also, antibodies to MS have been discovered in 90% of patients during incipient subclinical TB. Mtb malate synthase is an 741 amino acid monomer. Several (substrate-, inhibitor-, and product-bound) of MS have been reported, providing the atomic level details of the MS active site as well as the binding modes of MS ligands. Using this structural data, inhibitors can be designed to inhibit the activity of MS by exploiting its defined molecular structure. The potential inhibitor identified, this will be accomplished via a multifaceted approach: First, the inhibitor will be tested in vitro against Mtb MS to determine its potency against the enzyme. Second, compounds that have shown promising inhibition in vitro will be assayed for growth inhibition against whole cells (M. smegmatis) [13-20].

II. ENZYME INHIBITORS AND TUBERCULOSIS

A. Malate Synthase Inhibitor:
As the isocitrate lyase (ICL), the malate synthase (MS) is an enzyme of the glyoxylate shunt, a metabolic pathway of Mtb that appears to be upregulated during the chronic stage of infection. Since MS and ICL are part of the same metabolic pathway, inactivation of MS is expected to result in survival defects phenotypically similar to that observed in icl mutants. Identification of inhibitors for ICL has proven lengthy and laborious due to the conformation of the enzyme active side. Therefore, efforts are currently being focused on identifying inhibitors of the second identified component of the glyoxylate shunt, the MS. MS enzyme pathway that becomes heavily utilized and up regulated is the glyoxylate shunt. Since the glyoxylate shunt enzymes are not present in mammals, they make attractive drug targets [21].

B. Proteasome Inhibitors:
The proteasome (i.e. the protein degradation machinery of the cell) represents an interesting potential new target for anti-TB drugs. The activity of Mtb proteasome, appears to be important for protecting the bacteria from the killing effect of the nitric oxide produced in activated macrophages. The deletion of genes that encode proteins involved in the formation of proteasome causes hypersensitivity of the bacilli to nitric oxide. Drugs targeting the proteasome are expected to be active against MDR-TB strains as they would act through a completely novel mechanism.

C. Protease Inhibitors:
Proteases are a special class of proteins, operating as extremely precise biological 'scissors'—cutting long protein chains, thereby increasing or decreasing a particular protein's activity. Since 2001, the new antibiotics for identifying and validating bacterial proteases as target enzymes for pharmaceuticals. As part of this project, the genome of Mtb is being examined to identify genes encoding for proteases of interest as drug targets, to the purpose of developing protease inhibitors as antibiotics against these bacteria.

D. Phospho-N-acetylmuramyl-pentapeptide translocase Inhibitors:
Capuramycin analogues have been shown to have selective antibacterial activity against mycobacterium [22]. These compounds inhibit the phospho-N-acetylmuramyl-pentapeptide translocase and therefore interfere with the biosynthesis of mycobacterial the cell wall. When tested against Mtb, Capuramycins analogues appear to be equally active against drug susceptible and drug resistant strains. However, the MIC range showed by these compounds was significantly higher than the MIC for rifampicin (RIF) and isoniazid (INH) when tested against drug-susceptible strains [23]. Further studies are required to thoroughly assess the activity of the capuramycins analogues in vivo.

E. Topoisomerase Inhibitors:
The DNA Topoisomerases are enzymes that control DNA topology and are vital for cellular processes that involve duplex DNA, namely replication, recombination and transcription. DNA-gyrase, the single type-II topoisomerase of M. tuberculosis, is the molecular target of fluoroquinolones. Due to limited public information about this project, it is not known whether the new compounds under development will target DNA gyrase or DNA topoisomerase-I, the MDR-TB is a form of TB resistant to at least the two principal first-line drugs RIF and INH. The XDR-TB as a form of TB resistant not only to RIF and INH, but also to certain second-line drugs (kanamycin, amikacin or capreomycin).

F. ATP Synthase Inhibitor:
FAS20013 is a novel compound. It belongs to the class of 8-sulphonylcarboxamides. FAS20013 kill more organisms in a
4-hour exposure than INH or RIF can during a 12- to 14-day exposure. The compound is very effective in killing MDR-TB organisms that are resistant to multiple drugs currently in use. A series experiments indicates the superior effect of FAS20013 compared to current drugs in terms of its ability to "sterilize" TB lesions and kill latent TB. Therapeutic evaluation of FAS20013 has repeatedly shown its effectiveness in mice with no serious side effects. The compound is up to 100% bioavailable when administered orally. To date no dose-limiting toxicity has been encountered, even when doses 10 times the effective dose were administered. The compound is thought to act through inhibition of ATP synthase [24,25].

III. DISCUSSION

The immediate responses of the public health community must not focus solely on strengthening control programmes. It is also urgent to mobilise all necessary resources for the rapid delivery of new drugs and diagnostic tools that act through novel mechanisms which are able to target novel molecular targets, in order to avoid cross-resistance with drugs currently in use. Currently, there are a few new promising candidate drugs in the clinical phase of development. These candidate drugs have been shown to be active against MDR-TB strains in vitro and therefore have the potential to be effective against MDR-TB in human patients. There is an urgent need for innovative thinking in the field of clinical trials for new TB drugs, in order to speed up the development of these new drugs and accelerate their delivery to patients. A major limitation currently is the difficulty of diagnosing patients with TB. This problem is even more acute in the case of XDR-TB because the disease is so rapidly fatal that most patients will die before the results of their diagnosis are available. Rapid, reliable and field adapted diagnostic tools for TB and drug resistant forms of TB are an integral part of treatment strategies and urgently need to be developed. The combination of XDR-TB and HIV infection leads patients to develop a highly aggressive form of tuberculosis that causes death in a very short time. The emergence and rapid spread of XDR-TB in high HIV prevalence settings represent a major threat to global health. The phenomenon is a demonstration of the limitations of TB control programmes, which have been relying on outdated tools for TB diagnosis and treatment.

IV. CONCLUSIONS

The Mtb persistence enzyme, malate synthase, Proteasome, Protease, Phospho-N-acetylmuramyl-pentapeptide translocase, Topoisomerase and ATP Synthase has been studied with various biochemical, computational, and cellular approaches. These studies were initiated because of attractiveness of Mtb MS as a potentially novel anti-TB drug target. Not only is the enzyme MS not found in mammals, but attempts to knockout Mtb MS have been unsuccessful, indicating a possible essential role to Mtb. The discovery of a potent inhibitor against MS would help to validate the essentiality of MS. These studies have identified several inhibitor families. The discovery of a potent inhibitor of Mtb MS through biochemical, computational, and cellular approaches would lead to refinement of that hit into a "lead" compound. Lead compounds are then subjected to animal studies as a measure of their toxicity and efficacy. Progress to this stage leaves hope for the development of an anti-TB drug against the persistent Mtb via inhibition of MS.

REFERENCES


