NEW POTENTIAL TARGETS FOR FIGHTING AGAINST MULTI-DRUG RESISTANT TUBERCULOSIS

Ashish Sethi1, Sachdev Yadav2*, Rakesh kumar Sharma1
2. Assistant Professor (Pharmacology), Department of Pharmacy, Banasthali University, Rajasthan, India.

*Corresponding author’s E-mail: sachdev_y@yahoo.com

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ABSTRACT
Tuberculosis has re-emerged as a serious public health threat worldwide because of a significant increase in multiple drug resistant Mycobacterium tuberculosis varieties and a synergy between HIV and M. tuberculosis infection. It has been over 40 years since a new drug for tuberculosis has been discovered. The increasing emergence of drug resistant TB, and HIV infection, which compromises host defense and allows latent infection to reactivate or render individuals more susceptible to TB, pose further challenges for effective control of the disease. Although TB can be cured with chemotherapy, the treatment is exceedingly lengthy and takes 6–9 months. There is now recognition that new drugs to treat TB are urgently required, specifically for use in shorter treatment regimens than are possible with the current agents and which can be employed to treat multidrug-resistant and latent disease.

Keywords: Tuberculosis, Multidrug resistant, XDR TB, New Targets.

INTRODUCTION
Mycobacterium tuberculosis is transmitted through the air and infects about one-third of the world’s population. It often remains latent, and causes no harm to 90 percent of those infected. But more than nine million people each year develop active cases of TB, which typically causes weight loss, night sweats, and lung damage. Nearly two million people die each year from what should be a curable disease, either because they are not properly diagnosed, do not take the right medications, or have drug-resistant strains that defy treatment1. Nowadays, tuberculosis (TB) is becoming a worldwide problem. This contagious disease is transmitted through the air and it is caused by the bacterium Mycobacterium tuberculosis, which can attack different organs of human body. However, it most commonly affects the lungs, which is responsible for more than 75 percent of cases2.

Tuberculosis has re-emerged as a serious public health threat worldwide because of a significant increase in multiple drug resistant Mycobacterium tuberculosis varieties and a synergy between HIV and M. tuberculosis infection. It has been over 40 years since a new drug for tuberculosis has been discovered3. The increasing emergence of drug resistant TB, and HIV infection, which compromises host defense and allows latent infection to reactivate or render individuals more susceptible to TB, pose further challenges for effective control of the disease. Although TB can be cured with chemotherapy, the treatment is exceedingly lengthy and takes 6–9 months. Apart from significant toxicity, the lengthy therapy also creates poor patient compliance, which is a frequent cause for selection of drug resistant and often deadly multidrug resistant TB (MDR-TB) bacteria4. There is now recognition that new drugs to treat TB are urgently required, specifically for use in shorter treatment regimens than are possible with the current agents and which can be employed to treat multidrug-resistant and latent disease.

STATUS OF CURRENT TUBERCULOSIS DRUG THERAPY
Drugs available for the treatment of tuberculosis can be classified into two categories; first line drugs such as, isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), ethambutol (EMB) etc., and second line drugs like para amino salicylate (PAS), kanamycin, cycloserine (CS), ethionamide (ETA), amikacin, capreomycin, thiacetazone, fluoroquinolones etc.

Current TB therapy, also known as DOTS (directly observed treatment, short-course) consists of an initial phase of treatment with 4 drugs, INH, RIF, PZA and EMB, for 2 months daily, followed by treatment with INH and RIF for another 4 months, three times a week. The targets of these drugs are varied. INH, inhibits synthesis of mycolic acid, a cell well component; PZA targets cell membrane whereas rifampin and streptomycin interferes with the initiation and streptomycin interferes with the initiation of RNA and protein synthesis respectively. EMB blocks biosynthesis of arabinogalactan, a major polysaccharide present in the mycobacterial cell wall and kanamycin and capreomycin, like streptomycin, inhibit protein synthesis through modification of ribosomal structures at the 16S rRNA 13. Cycloserine prevents the synthesis of peptidoglycan, a constituent of cell wall6.
PROBLEMS WITH CURRENT TUBERCULOSIS TREATMENT

There are several major problems associated with the currently available TB treatment:

1. The duration and complexity of treatment result in nonadherence to treatment. This leads to suboptimal response (failure and relapse), the emergence of resistance, and continuous spread of the disease.

2. Adverse events in response to anti-TB drugs are common and contribute to the problem of nonadherence.

3. The increasing incidence of multidrug-resistant (MDR; resistance to at least rifampin and isoniazid) and extensively drug-resistant (XDR; MDR resistance plus resistance to a fluoroquinolone and an aminoglycoside) TB is a serious concern.


5. Prophylactic therapy of latent TB (TB infection without symptoms) with isoniazid is also associated with problems of nonadherence. Attempts to shorten treatment with alternative drugs resulted in severe adverse events.

Clearly, there is an urgent need to improve treatment by either enhancing the application of existing agents or introducing new drugs. Potential new agents should reduce treatment duration, have an acceptable tolerability profile, be active against MDR/XDR TB, be of use in HIV-infected patients with TB, and be active against latent TB.

NEED FOR NEW TUBERCULOSIS DRUGS

There are three reasons usually given for needing new tuberculosis drugs:

1. To improve current treatment by shortening the total duration of treatment and/or by providing for more widely spaced intermittent treatment.

2. To improve the treatment of MDR TB.

3. To provide for more effective treatment of Latent Tuberculosis Infection (LTBI) in programs those are able to implement this practice.

Table 1: Available and in Development.

<table>
<thead>
<tr>
<th>Anti-tuberculosis drugs in use</th>
<th>Date first used</th>
<th>Anti-tuberculosis drugs under development</th>
<th>New drugs in pre-clinical development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin</td>
<td>1945</td>
<td>Moxifloxacin (fluoroquinolone)</td>
<td>PA-824 nitroimidazole (TB Alliance, Chron)</td>
</tr>
<tr>
<td>Para-aminosalicylate (PAS)</td>
<td>1946</td>
<td>Gatifloxacin (fluoroquinolone)</td>
<td>OFC-6768 nitroimidazole (Otsuka)</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>1952</td>
<td>Linezolid (oxazolidinone)</td>
<td>TMC-207 dicyclafloxine (Tibotec)</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>1965</td>
<td>Metronidazole (nitroimidazole)</td>
<td>SQ-119 ethambutol derivative (sequlla)</td>
</tr>
<tr>
<td>Cycloserine</td>
<td>1965</td>
<td></td>
<td>LL-3658 pyrrolide (Lupin)</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>1968</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capreomycin</td>
<td>1969</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifampin</td>
<td>1967</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifapentine</td>
<td>1999</td>
<td></td>
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</tbody>
</table>

*Approved for human use in other infections. *Intermittent use once or twice per week only.

Table 2: Required Properties of New Anti-TB Drugs.

<table>
<thead>
<tr>
<th>What a new drug should do</th>
<th>Characteristic(s) required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simplify treatment or reduce treatment duration</td>
<td>Strong (early) bactericidal and sterilizing activity</td>
</tr>
<tr>
<td></td>
<td>Low pill count, fixed-dose combinations</td>
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<td></td>
<td>Allow for intermittent therapy</td>
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<tr>
<td>Have an acceptable toxicity profile</td>
<td>Low incidence of treatment-limiting adverse events</td>
</tr>
<tr>
<td>Be active against MDR/XDR TB</td>
<td>No cross-resistance with first-line drugs</td>
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<tr>
<td>Be useful in HIV-infected patients with TB</td>
<td>Minimal interactions with antiretroviral drugs</td>
</tr>
<tr>
<td>Be active against latent TB</td>
<td>No overlapping toxicity profile with other TB drugs</td>
</tr>
<tr>
<td></td>
<td>Activity against dormant bacilli</td>
</tr>
<tr>
<td></td>
<td>Favorable toxicity profile</td>
</tr>
</tbody>
</table>
CHALLENGES OF DEVELOPING NEW ANTI-TB DRUGS

The rapid development of new anti-TB drugs has been hampered by several obstacles.

- First of all, the TB drug market is associated with insufficient profit opportunity or investment return to instigate pharmaceutical industries to develop new drugs. The cost of developing a new drug is estimated at $115 to $240 million\textsuperscript{15}. To be profitable, market prices of new drugs should be relatively high, whereas the cost of the standard regimen is only about $11 per patient\textsuperscript{4}.

- Second challenge in TB drug development is the difficulty to identify new compounds with activity against M. tuberculosis. Regimens against TB should kill both the rapidly growing mycobacteria (bactericidal activity) and the persisting mycobacteria in lesions (sterilizing activity)\textsuperscript{7,16}. The molecular mechanisms responsible for mycobacterial dormancy (mycobacteria in a state of low metabolic activity and not forming colonies), persistence (drug-susceptible mycobacteria that manage to survive despite continuous exposure to TB drugs), and drug resistance are not yet fully understood\textsuperscript{10}.

- A next challenge rises with the evaluation of new compounds, as there are currently no animal models available that predict with accuracy the required treatment duration with newly identified compounds\textsuperscript{7,17}. The guinea pig model is being explored as an alternative for the mouse model since it resembles TB pathology in humans more closely\textsuperscript{7,18}.

- Another challenge is the scarcity of trial sites with sufficient research capacity to conduct clinical trials with large sample sizes. Trials should be performed in countries where the TB burden is highest, but the human and infrastructural capacity for performing large, high-quality phase III clinical trials is usually limited in these settings\textsuperscript{19}.

NEW TARGETS

GlgE

Despite vaccinations, screening, and antibiotics, tuberculosis remains the most deadly of bacterial infections. This global burden is due in part to the rise of drug-resistant strains of Mycobacterium tuberculosis, the bacterium that causes tuberculosis. However, the development of new treatment options requires a better understanding of this pathogen and how it causes disease. In Nature Chemical Biology, William Jacobs and colleagues propose a new approach to destroying tuberculosis – by manipulating the bacteria’s own metabolic pathways.

In Mycobacterium, cell wall and capsule components are generated through a four-step pathway in which the sugar trehalose is converted to α-glucan. Each step is catalyzed by a different enzyme. One of these, GlgE, is a maltosyltransferase that converts the intermediate compound maltose 1-phosphate (M1P) to α-glucan. Since the authors were unable to directly delete the GlgE gene in wildtype M. tuberculosis, they hypothesized that the enzyme is required for bacterial survival\textsuperscript{20}.

To further examine this possibility, they used a back-door method to generate a GlgE-deficient mutant strain. By deleting GlgE in a mutant M. tuberculosis strain already lacking TreS, the first enzyme in the trehalose-converting pathway, live bacteria could be obtained. Then TreS was re-introduced on a plasmid in the presence of an inhibitor. With removal of the TreS inhibitor, a surrogate GlgE knockout strain was born\textsuperscript{20}.

The GlgE surrogate knockout strain was then used to infect mice. Without the enzyme, the bacteria died rapidly in vivo, suggesting that the GlgE-mediated trehalose pathway is necessary for growth in the host. Since the enzyme wasn't present to convert M1P to α-glucan, the intermediate sugar accumulated to lethal concentrations in the bacteria. The basis for M1P toxicity was revealed by global gene expression analyses: excessive M1P damages bacterial DNA and leads to breakdown of cellular respiratory mechanisms. GlgE is absolutely required for M. tuberculosis survival. However, some bacteria were able to survive these experiments, probably by circumventing M1P accumulation with loss-of-function mutations in the TreS gene or another enzyme in the pathway.
Figure 2: A New Prokaryotic Pathway from Trehalose to A-Glucan. The reactions involved in conversion of trehalose to α-glucan comprise two essential steps in Mtb, catalyzed by GlgE and GlgB. \( n \) = number of glucose units\(^{20}\).

Given that survival of even a few bacteria limits the value of GlgE as a drug target, the authors also explored another pathway that \( M.\) \( \text{tuberculosis} \) uses to generate α-glucan. They found that one of the enzymes in this secondary pathway, the glucosyltransferase Rv3032, could not be inactivated in the mutant \( M.\) \( \text{tuberculosis} \) strain lacking TreS, demonstrating that these pathways are jointly essential. This timely study demonstrates a two-pronged approach for battling tuberculosis. By inactivating both GlgE and Rv3032, \( M.\) \( \text{tuberculosis} \) bacteria are unable to survive and the chance of selecting for resistant bugs is negligible. Furthermore, as neither humans nor beneficial gut bacteria express GlgE homologues, the side effects of anti-GlgE treatment would most likely be minimal. These results present a new understanding of the molecular mechanisms that drive bacterial pathogenesis and survival, thus providing important clues in the hunt for new \( M.\) \( \text{tuberculosis} \) drug targets\(^{20}\).

**LipB**

Several multicomponent enzyme complexes that catalyze key metabolic reactions in the citric acid cycle and single-carbon metabolism are posttranslationally modified by attachment to lipoic acid\(^{21}\). Growth of \( \text{Mycobacterium} \) \( \text{tuberculosis} \) relies on a pathway in which the lipoate attachment group is synthesized from an endogenously produced octanoic acid moiety. In patients with multiple-drug-resistant \( M.\) \( \text{tuberculosis} \), expression of one gene from this pathway, lipB, encoding for octanoyl-[acyl carrier protein]-protein acyltransferase is considerably up-regulated, thus making it a potential target in the search for novel antituberculosis agents\(^{22}\).

Figure 3: Structure-Based Proposal for a LipB Reaction Mechanism by means of a Covalent Cys-176–acyl intermediate\(^{22}\).
PEPCK

Metabolic adaptation to the host niche is a defining feature of the pathogenicity of Mycobacterium tuberculosis (Mtb). In vitro, Mtb is able to grow on a variety of carbon sources, but mounting evidence has implicated fatty acids as the major source of carbon and energy for Mtb during infection. When bacterial metabolism is primarily fueled by fatty acids, biosynthesis of sugars from intermediates of the tricarboxylic acid (TCA) cycle is essential for growth. Fatty acids are substrates for both the tricarboxylic acid (TCA) cycle and gluconeogenesis, which serve energy and biomass production, respectively. When biomass production solely relies on fatty acids, cells must avoid carbon loss in the form of CO2 during oxidation in the TCA cycle. This carbon preservation need is fulfilled by the glyoxylate cycle, which diverts isocitrate to succinate and glyoxylate through the joint activities of isocitrate lyase and malate synthase\(^ {23}\).

ClpP

The ATP-dependent Clp protease of Mycobacterium tuberculosis CDC1551 consists of two subunits, the ClpP subunit which is capable of degrading peptides in energy-dependent manner and ClpX, which possesses ATPase activity and activates the proteolytic activity of ClpP in vitro. ClpP is a core unit of a major bacterial protease complex implying as a new drug target for that isolates, which are resistant to antibiotics. ClpP is usually tightly regulated and strictly requires a member of the family of Clp-ATPase and often further accessory proteins for proteolytic activation. Binding of inhibitor with ClpP eliminates these safeguards. The inhibitor-activated ClpP core is capable of proteolytic degradation in the absence of the regulatory Clp-ATPases. Such uncontrolled proteolysis leads to inhibition of bacterial cell division and eventually cell death\(^ {24}\). ClpP is a key catalyst in proteolytic activation and a promising attractive drug target for novel antibiotics.

PD1 turns down the heat

Programmed death 1 (PD1; also known as PDCD1) is a costimulatory receptor found on immune effector cells that attenuates T cell activation and is known to be involved in the immune response to infection, particularly chronic viral infection. Two groups now show that PD1 also has a key role in regulating the innate immune response to Mycobacterium tuberculosis infection. PD1 is expressed by a range of immune effector cells, including T cells, natural killer (NK) cells and monocytes. Lázár-Molnár et al. investigated the function of PD1 in M. tuberculosis infection using a mouse model. The survival of PD1-deficient mice was markedly reduced after aerosol infection with M. tuberculosis. Histopathological examination revealed that PD1-deficient mice had a
significantly higher bacterial burden than wild-type mice and suffered severe multifocal necrotic pneumonia. Why are PD1-deficient mice so sensitive to M. tuberculosis? The authors examined the immune response to M. tuberculosis infection in these mice and found evidence for earlier recruitment of macrophages and neutrophils and higher levels of pro-inflammatory mediators than in wild-type mice. A detailed micro array analysis of the inflammatory response showed that more than 350 genes were differentially regulated in the lungs of PD1-deficient mice, with most of the upregulated genes encoding pro-inflammatory cytokines and chemokines that attract granulocytes. The authors conclude from their study that PD1 controls the inflammatory response to M. tuberculosis. Alvarez et al. reached the same conclusion from their analysis of the role of PD1 on NK cells in patients with tuberculosis. They present a model in which M. tuberculosis infection upregulates the expression of PD1 and PD1 ligands by NK cells. This stimulates the NK cells to lyse M. tuberculosis-infected cells, releasing interferon-γ (IFNγ), which in turn upregulates PD1 expression. Interactions between PD1-expressing cells then allow modulation of the innate immune response to M. tuberculosis infection. Thus, PD1 has a key role in inhibiting the immune response to M. tuberculosis infection in order to maintain control over inflammation. Blockade of the PD1 pathway has been pinpointed as a potential immunotherapeutic strategy to treat chronic viral infections, and Lázár-Molnár et al. caution that M. tuberculosis co-infection could be a confounding factor in such therapies.

CONCLUSION

The problem of pharmaceutical companies in developing a new drug for TB and the developed MDR TB can be resolved by taking into consideration any one of the proposed targets. So, we hope that in future a promising drug could be developed by targeting the proposed targets and will be able to eliminate TB completely from the society.

REFERENCES


23. Marrero J., Rhee K.Y., Schnappinger D., Pethe K., and Ehrt S., Gluconeogenic carbon flow of tricarboxylic acid cycle intermediates is critical for Mycobacterium tuberculosis to establish and maintain infection. PNAS 2010


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