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Review

Cell wall: A versatile fountain of drug targets in *Mycobacterium tuberculosis*

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ABSTRACT

Tuberculosis is the leading infectious disease responsible for an estimated one and a half million human deaths each year around the globe. HIV-TB coinfection and rapid increase in the emergence of drug resistant forms of TB is a dangerous scenario. This underlines the urgent need for new drugs with novel mechanism of action. A plethora of literature exist that highlight the importance of enzymes involved in the biosynthesis of mycobacterial cell wall responsible for its survival, growth, permeability, virulence and resistance to antibiotics. Therefore, assembly of cell wall components is an attractive target for the development of chemotherapeutics against *Mycobacterium tuberculosis*. The aim of this review is to highlight novel sets of enzyme inhibitors that disrupt its cell wall biosynthetic pathway. These include the currently approved first and second line drugs, candidates in clinical trials and current structure activity guided endeavors of scientific community to identify new potent inhibitors with least cytotoxicity and better efficacy against emergence of drug resistance till date.

1. Introduction

Tuberculosis (TB) [1] continues to remain a major global health problem in humans and a leading cause of death worldwide killing 1.5 million each year [2,3]. It is one of the most common opportunistic infection affecting HIV-positive individuals and leading cause of death in people living with HIV (PLHIV) [4,5]. HIV modifies the clinical manifestation of TB thereby delaying its diagnosis and early treatment. Today, HIV-TB co-infection along with emergence of multidrug-resistant TB (MDR-TB) poses a serious global threat. A vision of transition from stopping TB to ending TB is turning bleak. Therefore, addressing the burden of HIV-TB co-infection and optimizing current TB chemotherapy for more rapid and effective treatment of MDR-TB are major challenges. Innovation in search of new and effective anti-TB drugs (ATD's) will play a crucial role in curbing TB more effectively [6].

Decades of research all around the globe has led to the discovery of new molecules with anti-TB potential that are being currently evaluated both in pre-clinical and clinical stages of drug development. Gene products involved in controlling vital aspects of mycobacterial structure and metabolism like cell wall biosynthesis, DNA replication, RNA synthesis, protein synthesis, energy and folate metabolism represent attractive drug targets in *Mycobacterium tuberculosis*. However, in the last four decades of TB drug discovery, only a handful of antibiotics against drug resistant forms of *M. tuberculosis* are currently implemented by WHO owing to its tough cell wall with least permeability. The low permeability of the cell wall appears to be vital for survival of mycobacteria within the host as mycobacterial porin proteins are inefficient in allowing the permeation of solutes and hydrophilic antimicrobials agent across the cell wall inside the cytosol [7]. Advances in understanding the biology of mycobacterium has highlighted the importance of *M. tuberculosis* cell wall architecture, its biosynthetic pathways and permeability to play a pivotal role in the discovery of new ATD's to curb the increasing incidence of drug resistance [8].

In this review, we have attempted to highlight the drug targets involved in cell wall biosynthesis, their inhibitors and current efforts of researchers in this direction to find answers to emerging drug resistance in the light of cell wall inhibition from discovery to present times. Herein, we first briefly describe the cell wall architecture of *M. tuberculosis* with an aim to depict the enzymes and constituents involved in biosynthetic pathway for possible interference by antibiotics. This is followed by detailed mechanism of action of various cell wall inhibitors discovered so far that target cell wall of *M. tuberculosis*. These include:

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Mycolic acid (MA) inhibitors (isoniazid, ethionamide and nitroimidazoles); Arabinofuranosyl transferases (AT) inhibitors (ethambutol and ethylenediamine); Decaprenylphosphoryl-D-ribose-2′-epimerase (DprE) or Arabinan synthesis (AS) inhibitors (benzothiazinones); Peptidoglycan synthesis inhibitors like capuramycin, cycloserine and carbapenems. Simultaneously, we have attempted to include the recent literature on structure activity relationship (SAR) guided attempts of various research groups against each of the above mentioned drugs in improving them with respect to their potency, bioavalibility and safety profile.

2. History of development of tuberculosis chemotherapy

The timeline and evolution of TB drug discovery, development and evaluation has been fascinating [9]. The first antibiotic Streptomycin (STM) isolated from *Streptomyces griseus* was discovered almost 70 years ago. It displayed potential activity against *M. tuberculosis* thereby providing the first hope of TB specific chemotherapy [10,11]. Although in the beginning, small observational studies of streptomycin in human TB were encouraging, however its ability to consistently cure the patients remained uncertain due to rapid development of resistance against it. This led to the realization there was always danger of developing rapid drug resistance when a single agent is used in treatment of TB. In United Kingdom, Medical Research Council (MRC) TB unit was launched in 1946. They conducted the first recorded randomized, controlled clinical trial that was designed to compare streptomycin in combination with bed rest versus bed rest alone [12]. Their study revealed that administration of streptomycin followed by continuous bed rest achieved greater clinical improvement. However, the pathological improvement as assessed by chest radiography was only modest in comparison to bed rest alone. More importantly, although improvement in TB symptoms was greatest for first 3 months of therapy but lasted soon after which the condition of many patients began to deteriorate owing to the emergence of resistance to streptomycin. With the onset of 1950s, many other TB drugs with unique mechanisms of action were discovered and developed for TB treatment (Fig. 1). These discoveries paved the way for developing combination regimens that had very long treatment duration lasting for complete 18 months at that particular time. The next four decades were consumed in developing the current short-course therapeutic regimen by United States Public Health Service (USPHS) in collaboration with MRC-TB unit. The discovery of rifampicin in the 1960s was a major breakthrough that shortened the treatment duration to about 9 months in clinical practice. Finally when pyrazinamide was included in a regimen, the treatment duration was further decreased to only about 6 months.

The current globally recommended chemotherapy for the treatment of drug-susceptible TB (DS-TB) involves an intensive phase of four first-line ATD's: Rifampin (RIF), Isoniazid (INH), Pyrazinamide (PYZ) and Ethambutol (EMB) administered for the first 2 months followed by a continuation phase of RIF and INH for the next 4 months under directly observed treatment short course (DOTS) strategy [13]. Though DOTS strategy is labour intensive but it ensures high rates of patient treatment, adherence and completion [14]. Treatment success rate of 85% or more for new cases are regularly reported by WHO [15]. Since 1995 up to 2009, 41 million lives were saved under DOTS and STOP TB strategy [16,17].

Second-line drug regimens recommended by WHO for the treatment of MDR-TB include fluoroquinolones (ofloxacin, ciprofloxacin, moxifloxacin, levofloxacin) [18], aminoglycosides (kanamycin, amikacin),capreomycin, cycloserine, *para*-aminosalicylic acid, and thioamides (ethionamide, prothionamide) [19]. Suitable drug regimens should be ideally chosen by a stepwise selection process across the five categories of ATD's on the basis of efficacy, resistantance, susceptibility), safety and cost. The duration of the intensive phase of treatment is at least 6 months when an injectable drug is included or 4 months after culture conversion. The continuation phase without the injectable drug prolongs until 18 months after culture conversion. These drugs require at least 3-fold longer duration of administration than first-line drugs resulting in toxic side effects and loss of adherence besides lack of availability [20,21]. As such, it is need of an hour to discover new drugs that could address the current challenges associated with the treatment of TB.

3. Need for new tuberculosis drugs

TB as a global agenda was utterly neglected for a period of at least four decades that generated a climate of indifference to the need for fresh drugs. As a result of this apathy, funding and interest for TB

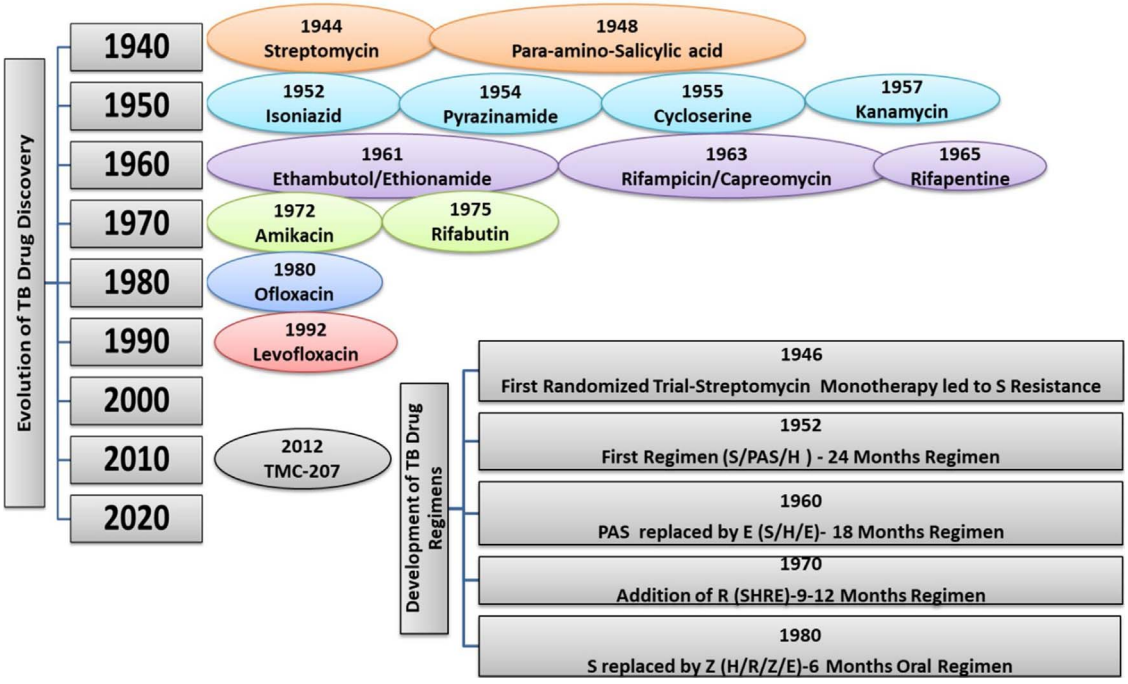

Fig. 1. History of TB drug discovery and development of TB drug regimens.