TUBERCULOSIS: PATHOGENESIS, EMERGING DRUG TARGETS, CURRENT DRUG REGIMENS AND DRUGS IN CLINICAL DEVELOPMENT

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ABSTRACT: Tuberculosis is a bacterial infection. It is the chronic infectious disease caused by the tubercle bacillius. In this paper we present a general overview of TB drug targets, diagnosis, medicinal plant treatment and new drugs in clinical development

KEY WORDS: Tuberculosis(TB), Drug-resistance, pathogenesis, diagnosis, medicinal plant treatment in tuberculosis.

INTRODUCTION

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* bacteria, which generally affects the lungs, but can also affect the other body parts such as kidney, spine, brain. TB bacteria are spread through air from one person to another person. The TB bacteria are spread in air when infected TB person sneeze in air. People may breathe these bacteria and become infected. The bacteria can settle in the lungs and being to grow. They can move through the blood to other parts of body. TB disease in the lungs or throat can be infectious. TB in other parts of body like kidney or spine is usually not infectious. The symptoms of active TB of lungs are coughing, blood in the sputum, fever, weight loss, night sweats[1]. The TB has now emerged as a global health crisis. Worldwide in 2017, 6.4 million new cases of TB notified[2]. This number has been increasing. As more anti-TB drug were discovered it was realized that combination of 2-4 anti-TB drugs used for longer periods as effective to kill the bacteria and prevent from resistance [3].

MICROBIOLOGY OF *M. TUBERCULOSIS*

Mycobacterium tuberculosis is a non-motile, rod-shaped, acid-fast, obligate aerobe, nonspore forming bacteria. The bacilli are 2-4µm in length and have very slow generation time between 15 and 20 hours. The cell wall of the mycobacterium is unique in that it is formed by acid waxes, specifically mycolic acids. *M. tuberculosis* is usually resistant to drying and chemicals, contributing to the ease with which it is transmitted[4]. The cell wall is made of carbohydrates, lipids and proteins. The alfa, keto and methoxy mycolic acid is important component of cell wall. Porins are present in cell wall through which substance can diffuse. Total 4000 genes present in mycobacterial genome. 6 and 8% of these are devoted to fatty acid metabolism. The remaining encodes are various enzymes and transcriptional regulators essential for bacterial growth and survival. These enzymes and regulators serve as mycobacterial virulence factors[5]. Histidine kinase, protein kinase and protein tyrosine phosphates are signaling enzyme essential for bacterial survival. Glycerol, citrate and asparagines are growth factor of mycobacteria[6]. Central carbon metabolism is essential for mycobacterial physiology, pathogenesis and survival.

PATHOGENESIS

M. tuberculosis is spread in air as droplet nuclei from coughing, sneezing, shouting from individuals with pulmonary or laryngeal TB. Transmission occurs when inhalation of these droplet nuclei pass through mouth or nasal cavity, the upper respiratory tract, bronchi and then reaches the alveoli of the lungs[7].once the tubercle bacilli reaches the alveoli, they are ingested by alveolar macrophages resulting in the destruction and inhibition of inhaled tubercle bacilli[8]. The small unaffected proportion multiplies within macrophages. Released of tubercle bacilli spread through the bloodstream or lymphatic channels to any part of the body tissues and organs and another highly susceptible area of TB infection such as the lungs, larynx, lymph nodes, spine, bone or kidney[9]. In about 2 to 8 weeks[10], an immune response is triggered which allows white blood cells to encapsulate or destroy majority of the tubercle bacilli. The encapsulation by the white blood cells resulting in barrier around the tubercle bacilli forming a granuloma[8]. Once inside the barrier shell, the tubercle bacilli is said to be under control and thus

establishing a state of latent tuberculosis infection[LTBI]. Person at this stage not show any TB related symptoms, are not spread the infection and are not consider as TB cases[11]. On other hand, if immune system fails to control the tubercle bacilli, rapid multiplication of the bacilli leads to a progression from LTBI to a case of TB. TB cases is highly infectious and can spread the tubercle bacilli to other people[12].

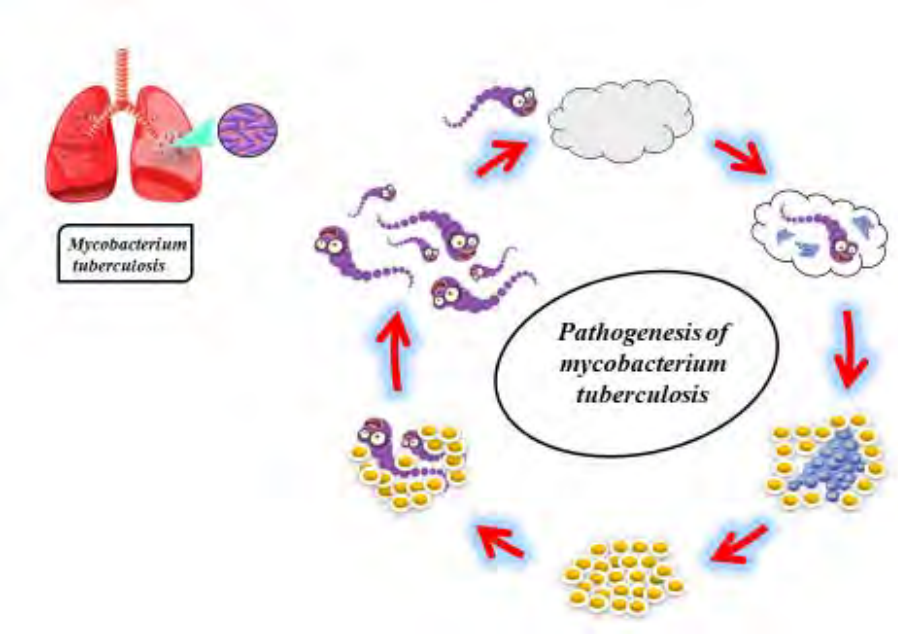


Figure1: Pathogenesis of mycobacterium tuberculosis

DRUG THERAPY AND DRUG RESISTANCE

Since the discovery of effective anti-TB drugs, the drug resistance is one of the most major challenge against TB. To avoid this problem, a number of measures and strategies are adopted for prevention and control of drug resistance. These include use of combination therapy, directly observed treatement, short course(DOTS) strategy[13,14] and its expanded form DOTS-Plus[15]. Some TB chemotherapeutics regimen has not show much change in last 50 years. The regimen includes isoniazid(INH), rifampicin(RMP), pyrazinamide(PZA), ethambutol(ETH) or streptomycin for 2 months followed by 4 months of isoniazide and rifampicin (Table1)[16]. Substandard drugs, irrational prescribing practices and nonadherence to complex regimens increase selection of drug-resistant strain of TB bacilli. This along with rise in HIV co-infection has undetermined the efforts of chemotherapy and increases the percentage of mycobacteria resistant to isoniazid and rifampicin (multidrug resistant TB, MDR-TB), and another additional resistance to fluoroquinolones and second-line TB drugs are also observed (extensively drug-resistance TB, XDR-TB). In additional, there are phenotypic persisters avoid the killing effects of chemotherapeutic drugs[17]. Twenty or more months of treatment with less effective, more toxic and costlier drug manages to cure only 60-75% of patients. It is essential to have safer and cheaper alternatives with novel mechanism of action and capability to be effective in monotherapy and dual therapy. Less frequent dosing and short TB therapy duration are unacceptable. The new drug are active against resistant TB pathogens and latent TB. They are kill rapidly multiplying bacteria[18]. In last few decades improvement of anti-TB drugs. Clinical trial approvals are faster. Newer drug delivery system are exploited to enhance drug bioavailability and reduce ill effects [19]. Older drug are used in different way for TB treatment and improved analogue of antimycobacterial drugs are manufactured[20].

SOME NEW EMERGING DRUGS TARGETS

Since the discovery of the complete genome sequence of a virulent strain of TB bacteria, lots of new target have been identified. The evaluation of new targets is essential to carry on the constant battle against the drug resistant strain of TB .

Cell wall targets

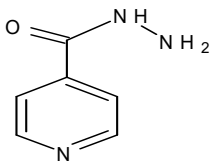
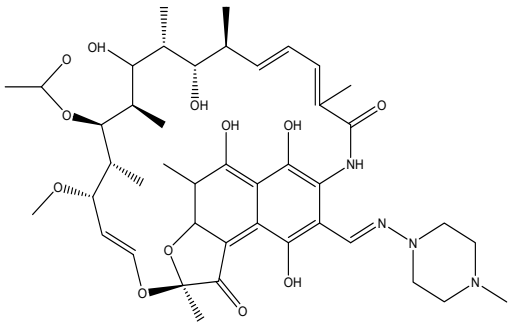
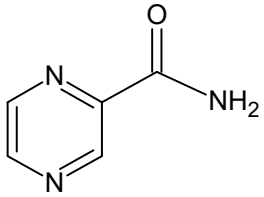
The mycobacterial cell envelope is more complex bacterial structures identified and major barrier for drug penetration . The cell envelope is covalently linked mycolic acids, d-arabino-d-galactan and peptidoglycan. The mycolic acid which are accompanied by glycolipids. The mycolic acids based on permeability barrier that shed

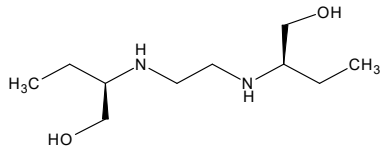
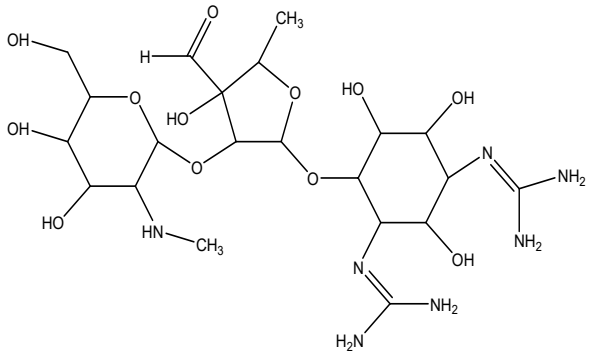
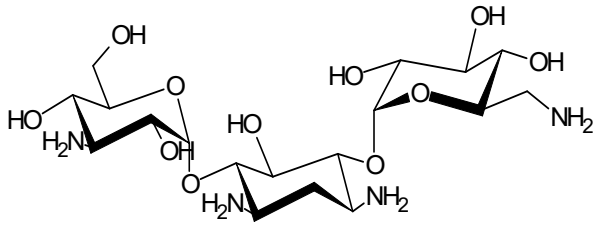
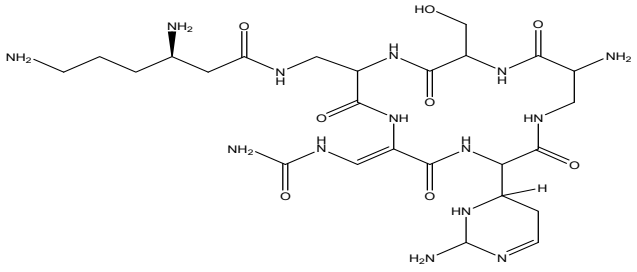
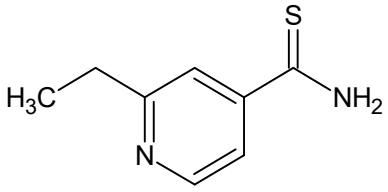
the organism from environmental stress, contributes to the disease persistence and the intractability of the *M. tuberculosis* to many antibiotics[21]. The essential genes responsible for the cell growth under all conditions are primary target to treat infectious disease. Novel and classic technique are essential cellular components for discovery of the new drugs and drugs targets[22]. The isoniazid and ethambutol are two first-line tuberculosis drugs inhibiting cell wall biosynthesis. The target based whole cell screen via the growth defective mutant can increase the sensitivity towards small molecule inhibitor[23]. The construction of the *M.tuberculosis* mutant that overexpress various genes like *LepB,PanC, Icll* and *LysA* for alteration of the specific affinity on variable target act as small molecule inhibitor. This approach is used for identify the inhibitor of the pantothenate synthase and diaminopimelate decarboxylase[24].

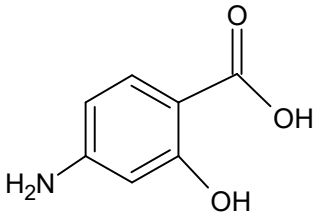
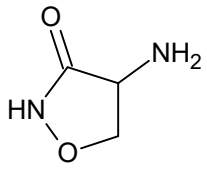
Bacterial targets

Now a day various bacterial target are found. These targets include various enzymes, genes controlling these enzymes and other proteins. Glycolytic pathway, electron transport chains and fermentation pathway holds as targets for anti-TB drugs. Sigma factors regulate the mycobacterial regulators during period of environmental stress and then inhibited in order to examine the persister[25].

Table1: The Summary of Different First Line and Second Line Drugs of TB

DRUG	MECHANISM OF ACTION	REMARKS
FIRST LINE DRUGS		
Isoniazid (INH) 	Inhibit the synthesis of mycolic acid	Effective against rapidly multiplying bacteria, it inhibits the cytochrome P450 system
Rifampicin(RMP) 	Inhibition of β subunit of RNA polymerase and nucleic acid synthesis	Acts on bacteria with metabolic spurts
Pyrazinamide(PZA) 	Depletes membrane energy	Acts in acidic media, bacteria with low metabolic rate are also killed

Ethambutol(ETH) 	Acts on cell wall arabinogalactan	Acts on rapidly proliferating bacteria, decrease INH resistance
Streptomycin 	Decreases protein synthesis	Injectable, nephro-and ototoxicity
SECOND-LINE DRUGS		
Kanamycin 	Decreases protein synthesis	Injectable, nephro-and ototoxicity
Capreomycin(polypeptide antibiotic) 	Decrease DNA synthesis	Injectable, nephro-and ototoxicity
Ethionamide 	Inhibition of mycolic acid synthesis	Oral, thyroid hormone inhibition

Para-amino salicylic acid 	Folate synthesis inhibition	The most common side effects of PAS are persistent nausea, vomiting and diarrshoea. If the patient experiences fatigue, it may be due to hypothyroidism caused by PAS. PAS may cause hepatitis occasional side effects include increased prothrombin time and malabsorption syndrome
Cycloserine 	Inhibition of cell wall peptidoglycan biosynthesis via action on D-alanine racemase and ligase	-

Nuclear targets

A bacterial tubulin polymerase inhibitors such as deazapteridine compounds includes SRI-3072, are inhibiting mycobacteria[26-28]. They act on cellular division. Purine salvage pathway is essential for growth and survival of bacteria. Purine nucleoside analogs targets to purine nucleosidase enzyme of this pathway to produce anti-TB action[29,30]. 9-Sulfonylated are potent and selective against mycobacteria. The potent 9-benzylpurines and arylpurines acts on intracellular *TB bacilli* [31].

DIAGNOSIS OF LATENT *M. TUBERCULOSIS* INFECTION

The person infected with *M. tuberculosis* may be identified by tuberculin skin test, six to eight weeks after exposure to the bacilli. This test based on delayed- type hypersensitivity (DTH) response to a *M. tuberculosis* antigens, called as purified protein derivative (PPD). Size of more than 5mm, recorded 48 to 72 hours after injection of PPD, is consider as positive[32]. Skin test reaction over 20mm is usually due to active disease; however, a negative skin test in an active TB patient may also result from anergy or incorrect administration of the test. The tuberculin skin test lacks sensitivity and it can not differentiate between infection with *M. tuberculosis*[32,33]. More sensitive and specific test is cell-mediated immunity- based interferon-gamma(IFN-γ) release assays ($IGRA_S$) have been developed to detect T cell responses after stimulation by two *M. tuberculosis*-specific antigens, early secreted antigenic target-6 (ESAT-6) and culture filtrate protein-10(CFP-10)[34-39]. Another variation of conventional $IGRA_S$ also developed by using cytometry[40]. The flow cytometric approach has more advantageous than conventional $IGRA_S$, small blood volume(<1ml) is needed for testing, the assay has limited utility in much of the developing world due to the requirement of technical expertise and high cost of flow cytometers. The detection of antibodies level to some *M. tuberculosis*-specific proteins has also been noted in latently infected individuals and patient with active TB disease[41,44]. Two commercial $IGRA_S$, whole blood, ELISA-based QuantiFERON (QFN)- TB Gold assay (Cellestis Ltd., Carnegie, Australia) and peripheral blood mononuclear cell(PBMC) and enzyme-linked immunospot (ELISPOT) technology based T SPOT-T B (Oxford Immunotec, Oxford, UK) has been developed for detecting LTBI and these test have been approved by Food And Drug Administration(FDA). These test is based on stimulation of T-lymphocytes with ESAT-6 and CFP-10 proteins and measurement of IFN-γ production (QFN-TB Gold) or detection of T-cells themselves (T SPOT-TB). The

newer version of the (QFN-G-IT) (Cellestis Ltd., Carnegie, Australia) uses ESAT-6 and CFP-10 and TB7. Peptides as antigens. The performance of both QFT-G-IT and T-Spot-TB tests have been evaluated extensively[35,45,46,47,48]. Although $IGRA_S$ can not distinguish between LTBI and active TB disease in immunocompetent adult, in high-risk individuals with immunosuppressive conditions and children, $IGRA_S$ may help in diagnosis of active disease as adjunctive diagnosis test, particularly if specimen from the suspected site of infection such as bronchoalveolar lavage, cerebrospinal fluid[49,50.]

IMPROVEMENT IN CURRENT DRUG REGIMENS

Development of new drug is difficult, time consuming and expensive process, efforts have been needed to improve on present regimens:

Improvement of regimens: Adherence to therapy is an problem and could be bettered by close monitoring for adversities and their prompt management[51]. Combining 2-4 anti-TB drugs into one formulation is to improve compliance.

New drug delivery techniques: The new drug delivery system with anti-TB drugs are effective to reach target tissue or cell and need less quantity of dosing and hence to improve patient compliance. Aerosolized form of anti-TB drugs can be deliver the active ingredient to the lungs. Global Alliance on TB(GATB) is currently evaluate safety, efficacy and advantages of this drug delivery system in TB management[19,52]. Liposome and bio-degradable polymer based technology are used for sustain delivery of conventional anti-TB drugs[52]. This leads to improved compliance and clinical outcome.

Supplementary therapy: The anti-TB drugs administered along with immunomodulators could enhance efficacy[18]. Adenine triphosphate (ATP) have been used to potentiate macrophage antimycobacterial activity[53]. Anti-TB drug with immunomodulator in suitable nanoparticles have been tried for better solubility and bioavailability[54,55].

NEW DRUGS IN CLINICAL DEVELOPMENT

Aim of finding new antituberculosis drugs are in progress and compounds showed novel Chemical structure and acts on novel targets[56-59].The drugs include, GSK 2556286;[60] TBAJ-587 from diarylquinoline;[60] TBI-223 from oxazolidinone;[61] spectinamide 1810 and spectinomycin analogues,[62] BTZ-043,[63] GSK 070 and GSK 3036656 from oxaborole;[60] contezolid (MRX-4/MRX-1) from oxazolidinone;[64] OPC167832, a 3,4-dihyrdocarbostyril derivative;[65] Macozinone (PBTZ169), a piperazinobenzothiazinone derivative;[66,67]clofazimine (TBI-166) from riminophenazine;[68] TBA-7371 from azaindole;[60] and Sutezolid from oxazolidinone.[69-72]

MEDICINAL PLANT TREATMENT IN TUBERCULOSIS

The importance of plants has been recorded since the ancient time due to virtuousness of its variety of chemical compounds, it contain some important medicinal properties that can be used to cure diseases. Medicinal plant have been different preventive and curative solution against disease[73-76]. The WHO estimated that about 80 percent of world population depend on traditional medicinal plants for their health care. The uses of herbs and herbal products have been accepted in our modern way of life[77]. Most of the drug cause adverse effects and costlier, therefore nowadays there are used of alternative source of medicine, usually based on medicinal plants[78]. The number of medicinal plant have been reported for anti-mycobacterial action[79-84]. *Allium sativum* (garlic) has been show to be antimicrobial and immunomodulating activity[85,86]. It contain allicin which inhibits glutathione peroxidise and hence, bacterial replication is reduced. Extracts of *Acalypha indica, Adhatoda vasica,Actiniopteris radiata, Aloe vera and Alstonia scholaris* were found to inhibit mycobacteria of TB[87,88]. Extracts of *Gycyrrhizza glabra, Cassia fistula, Quercus infectora, Strophanthus wallichii, Lawsonia intermis, Piper nigrum, Syzgium aromaticum*, *Acacia catechu, Vevtiveria zizanioides* also demonstrated the significant antimycobacterial activity[89-96]. Acetone extract of pulverized aerial portion of *Leucas steligera* contain diterpenes and flavones act as antimicrobial activity[97]. Methanolic extracts of *Duroia macrophylla* contain terpenes and flavonoids with antimycobacterial efficacy[98]. *Byttneria herbecea* extract can inhibit glutamine synthetase and kill both growing and dormant bacilli[99]. These plants are promising candidates to find novel medication for the treatment of TB. However, the emergence of MDR and XDR-TB has further inspired the scientific community to find novel and more potent anti-mycobacterial drug molecules. Various plants across the world possess anti-mycobacterial activity against MDR-TB. India is one of the leading countries in herbal medicines and researchers are continuously engaged in searching novel drug molecules to combat MDR/XDR-TB. since last few years several plants have been reported for their anti-mycobacterial activity from India.

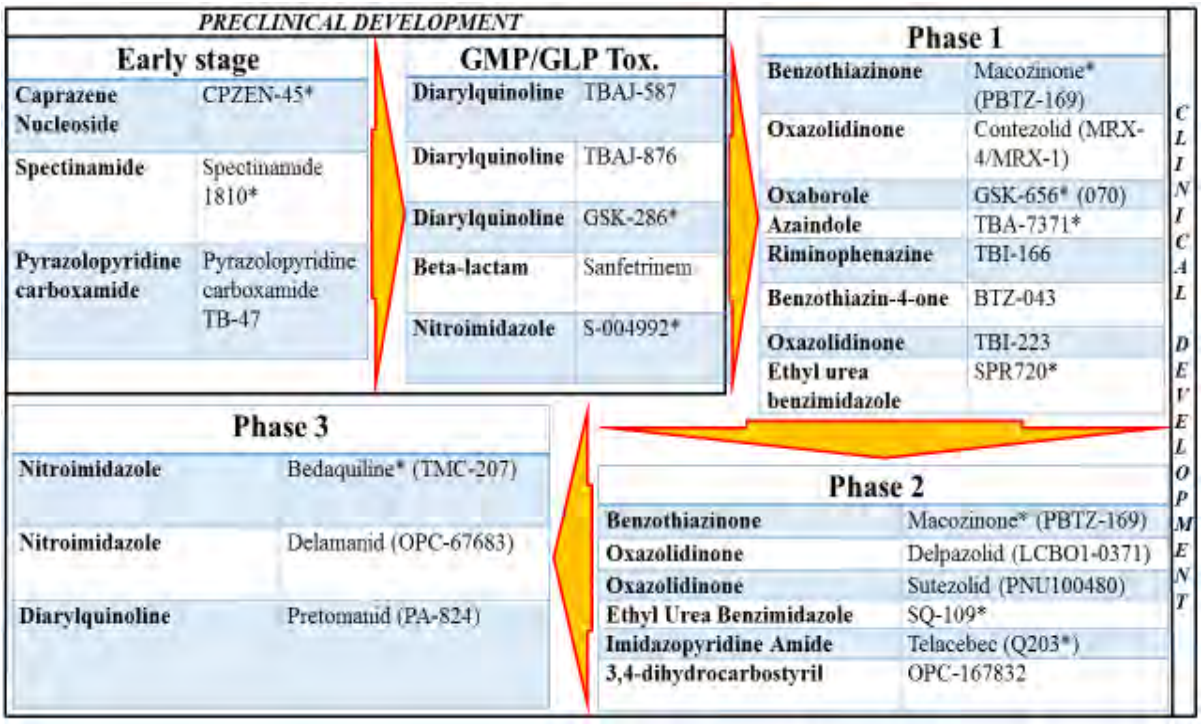


Table 2: Global new tuberculosis drug development pipelines

CONCLUSION

Tuberculosis is one of the most infectious disease and has claimed millions of lives for many years. Some new emerging drug targets and drugs has been identified to carry on the constant battle against the drug resistant strain of tuberculosis.

ACNOWLEDGEMENT

Authors are thankful to the Management and Principal of MGV's Pharmacy College, Panchavati, Nashik and Savitribai Phule Pune University, Pune.

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