Current Research in Microbiology

Chapter 6

Drug Resistant *Mycobacterium tuberculosis*

Parthiban Brindha Devi

Department of Bio-Engineering, Vels Institute of Science, Technology and Advanced Studies, Velan Nagar, Chennai, Tamil Nadu, India

Email: pbrindhadevi@gmail.coms

1. Introduction

Mycobacterium tuberculosis (Mtb) is a causative agent for the deadly pathogenic disease Tuberculosis. It was said earlier that around 10 million of new cases are being infected with tuberculosis [1]. This implies that there is minimizing in the treatment of tuberculosis. This is mainly due to the resistant strains being developed by the bacteria and the various forms of diseases have developed due to its resistance form. Tuberculosis is an air born infection which is highly transmitted by aerosol. In recent years, Tuberculosis caused by Mtb is emerging in a very faster rate by its resistant strains. Its been evolved as Multidrug resistant Tuberculosis and Extremely Drug resistant Tuberculosis. Recent studies have showed that the Mtb is fully resistant to all the antibiotics and its given as Totally Drug resistant Tuberculosis. Recently, world health organization has formed a global meet for treating tuberculosis and has made an urgent action to stop this disease by 2030. The mortality rate for tuberculosis is around 37% for past 10 years. However still there is many TB cases without any prevention or cure. It has also been reported that, TB is a deadly pathogenic diseases than any other infectious diseases. The main reason is antimicrobial resistance. Also it mainly affect the patient who is being suffering from HIV.

2. Mtb Pathogenesis

Around 1/3rd of world population is being infected with non-replicating or dormant Mtb. TB can affect any organs in the human body but mostly it affects the Lungs [4,5]. Mtb is transmitted by air and it reaches the pulmonary alveoli; once after reaching the alveolar region, phagocytosis process will start and the bacteria will phagocytized by non-activated

macrophages. Inside the macrophage, the bacteria inhibit many cellular mechanisms and prevent the fusion formation of phagosomes and lysosomes. The ability of the bacteria to circumvent phagolysosome degradation allows the bacteria to replicate freely and when the macrophage lyses the bacteria it starts invading the new macrophages [7]. When larger number of macrophages is infected, there forms a compartment call granuloma. During this state, the immune system suppresses the infection and the bacteria stops replicating and it enters into the dormant state within the granulomas. Suppression of immune system due to HIV or if the person reaches their old age the dormant state TB will reactivates into active form Tuberculosis and causes the disease. The pathogenic bacteria inside the granuloma can survive for a longer period of time and it develops hypoxic environment, Reactive oxygen intermediates, Reactive nitrogen intermediates and nutrient starved conditions. The mycolic acid which is present in the abundant form in the mycobacteria plays an important role in the dormant Tuberculosis. It is also been reported that, the lipids play the major energy source for persistence in the host. The Mtb bacteria use the host triacylglycerol to accumulate lipid droplets intracellularly and acquire dormancy phenotype inside the macrophage [10]. Recently it was reported that, dormant bacteria may be eradicated with antibiotics generated with hydroxyl radicals, suggesting that stimulation of reactive oxygen species provide potential strategy to manage persistent infections [6,7]. Figure 1 shows the pathogenesis of both active and dormant tuberculosis [15].

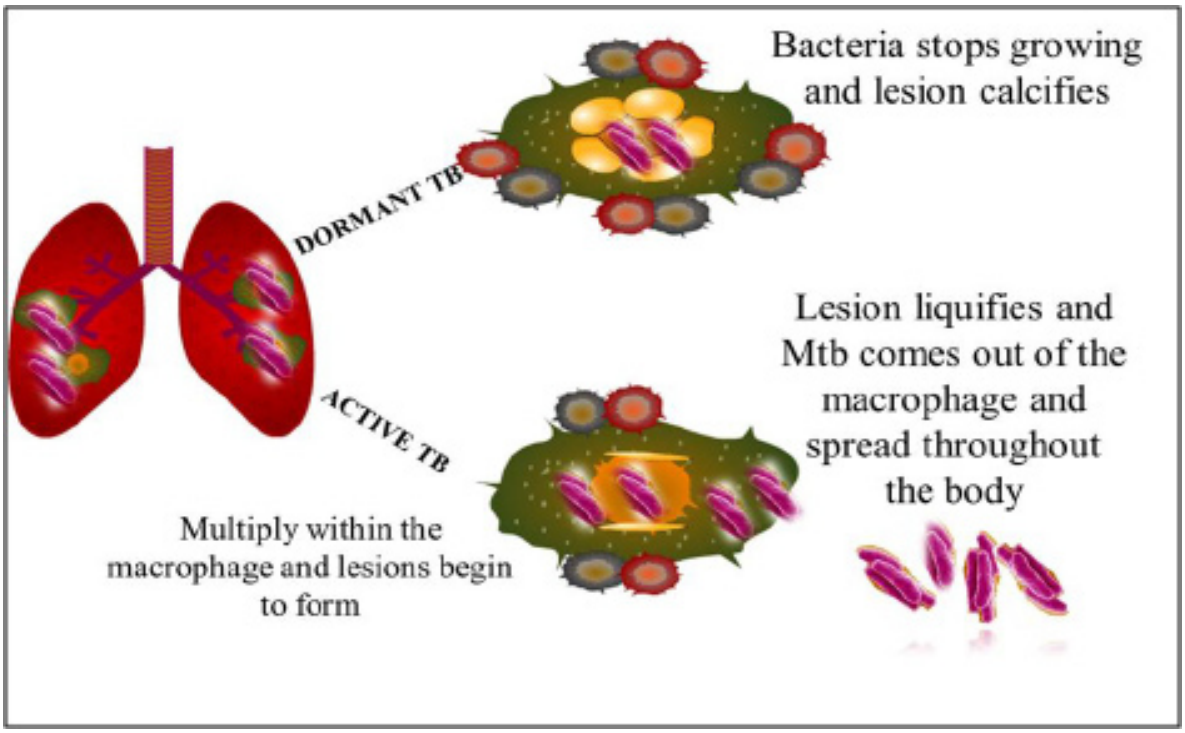

Figure 1: Pathogenesis of Mycobacterium tuberculosis

3. TB Chemotherapy

The drugs being used for tuberculosis is from the first line TB drugs discovered since in 1950's and 1960's. Streptomycin was the first antibiotic which is used as an effective antimycobacterial agent. The aminoglycoside group present in the streptomycin interferes with protein biosynthesis through the interaction with 30S ribosome subunit. In later years, Isoniazid,

mycolic acid synthesis inhibitor was one of the most active TB drugs till date. The discovery of pyrazinamide is a major breakthrough in TB treatment which helps in the bacterial load if the medication is taken for the period of 6 months to 9 months [12, 13]. In 1961, investigation of polyamines and diamines led to production of a series of diamine analogues that gave rise to discover of ethambutol [11]. Finally rifampicin (RIF) [1] the last member of present first-line drugs was found effective against replicating and non-replicating Mtb. This class of drugs inhibited RNA synthesis by binding to the B-subunit of DNA-dependent polymerase. Inspite of these efficient drugs, the bacteria is being mutated and started becoming resistant to all the drugs being reported and used for the treatment. Inorder to prevent the bacteria from developing resistance, combination therapy was introduced. World health organization has recommended direct observed treatment which is commonly called DOTS anti-TB therapy with the combination of four drugs: Isoniazid, Ethambutol and Pyrazinamide for two months and followed by Rifampicin and Isoniazid for Four months. This therapy worked well with the patients and the cure rate was increased by 90%. This has been accepted globally for active TB treatment. Treatment with these first-line drugs when carried for two months led to destruction of bacteria in all stages of growth and when the treatment continued with rifampicin and isoniazid for four months, residual dormant bacilli was eliminated using rifampicin and any rifampicin resistant mutant was killed by isoniazid.

4. Limitation of TB Drug therapy

The combination anti-TB therapy was quite acceptable globally but the treatment process is very expensive and has to be administered for a longer duration which led to significant side effects to the patients. The length of therapy makes the patient compliance difficult and these patients would become susceptible to drug-resistant strains. The second major problem was that the current therapies available today were ineffective against persistent bacilli except rifampicin and pyrazinamide. Rifampicin was active against both actively growing and slow metabolizing non-growing bacilli whereas pyrazinamide was active against semi-dormant non-growing bacilli. However there are still dormant bacilli populations not killed by any of the available drugs [Chopra P., *et al*., 2003]. Therefore, there is a need for new drugs that are more active against slowly growing and persistent bacilli to treat populations of bacteria at risk of developing active disease through reactivation. Also, it is important to achieve shortened therapy to slow down the development of drug resistance in mycobacteria.

5. Drug Resistant Tuberculosis

Multi drug resistant tuberculosis is resistant to first line drugs Isoniazid and Rifampicin. The term MDR-TB was associated with high mortality rates occurred among HIV-infected patients [WHO report, 2013]. The treatment is quite complicated and it requires second-line drugs some of which are less effective, more toxic and expensive than first-line drugs. XDR-

TB is resistant to at least isoniazid and rifampicin and also to fluoroquinolones, capreomycin, kanamycin and amikacin. XDR-TB requires longer treatments with drugs are very costly with limited efficacy and increased side effects. XDR-TB is reported in 77 countries and its prevalence is not clear. Only two of 27 burden MDR-TB countries are routinely testing for the resistance of second-line drugs; since XDR-TB is resistant to first and second-line drugs. The genetic diversity of drug-resistant Mtb indicates that the drug resistance has been evolving due to inappropriate drug treatment [Trauner A., *et al*., 2014]. The ongoing evolution of Mtb, provide excellent opportunity to explore genetic determinants of drugs resistance to Mtb [8].

Unlike other bacterial pathogens, resistance plasmids and horizontal gene transfer plays no role in drug resistance to Mtb. Efflux mechanism seem to play vital role in developing resistance to this pathogenic bacterium. Due to genetic mutations could be helpful for reliable molecular markers for drug susceptibility testing. The application of this technology to clinical research led to the development of diagnostic tool based on nucleic acid amplification [12].

6. TB drug pipeline

A major advance in the screening efficacy for novel targets was achieved by shifting from enzyme targets to phenotypic screening of whole bacterial cell. Whole bacterial cell screening identified diarylquinolones (bedaquiline), benzothiazines (BTZ-043 and PBTZ-169), and imidazopyridine amide (Q-203) [Zumla A.I., et al., 2014].

Bedaquiline (TMC-207), an adenosine triphosphate synthase inhibitor was recently approved by U.S. FDA for the treatment of MDR-TB as a part of new combination therapy. Also, IOC-67683 (delamanid) and TBA-354, a second generation nitroimidazole has also entered phase 3 trials for the treatment of MDR-TB.

Q-203, a compound made from imidazopyridines inhibits the mycobacterial growth by blocking respiratory cytochrome essential for maintaining the proton gradient and ATP synthesis. This drug also has similar property as bedaquiline and inhibits both replicating and non-replicating Mtb. It is active against MDR-TB, XDR-TB and in-vivo data shows there is 100-1000 fold reduction of CFU and blocking of granuloma formation.

Benzothiazinones derivatives, PBTZ-169 and BTZ-043 are in late stage of clinical development. Both drugs inhibits the enzyme, decaprenylphosphory-D-ribose 2’ epimerase (DprE1) in Mtb. Inhibition of this enzyme prevents the formation of decaprenylphosphoryl arabinose- a key precursor for biosynthesis of cell wall arabinans, resulting in cell lysis and bacterial death. Both the compounds show 100-1000 fold reduction in CFU *in-vivo* [13].

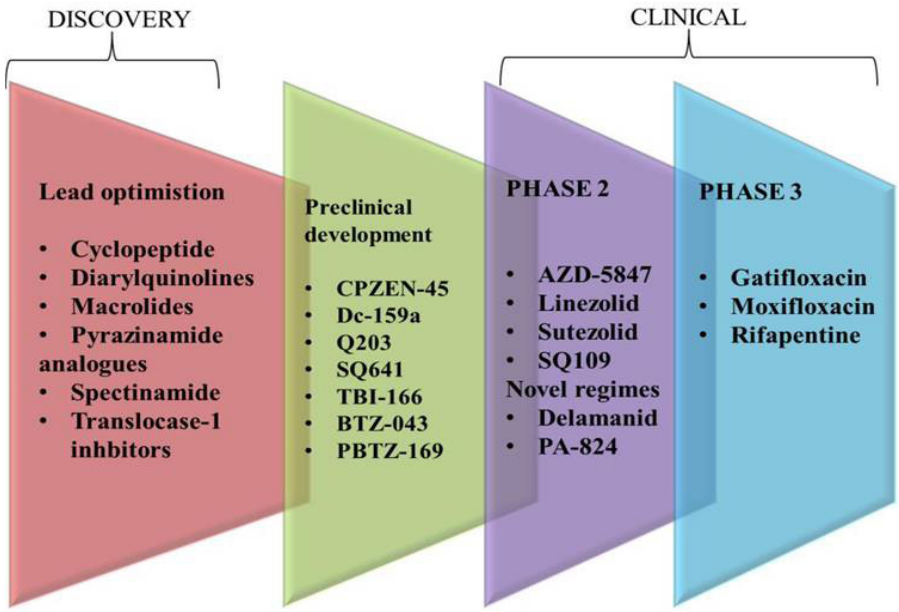

Figure 2: TB drugs pipeline

7. References

1. Binda G, Domenichini E, Gottardi A, Orlandi B, Ortelli E, Pacini B, ET AL. Rifampicin, a general review. Arzneimittelforschung. 1971; 12: 1907-1977.

2. Chopra P, Meena LS, Singh Y, New drugs for Mycobacterium tuberculosis, Indian J Med Res. 2003; 117: 1-9.

3. Cox HS, Ford N, Reeder JC, Are we really that good at treating tuberculosis?. Lancet Infect. 2009; 9: 138-139.

4. Elzinga G, Raviglione MC, Maher D, Scale up: Meeting targets in global tuberculosis control. Lancet. 2004; 363: 814-819.

5. Fattorini L, Piccaro G, Mustazzolu A, Giannoni F. Targeting dormant bacilli to fight tuberculosis, Mediterr J Hematol Infect Dis. 2013; 5: e2013072.

6. Graham JE, Clark-Curtiss JE, Identification of Mycobacterium tuberculosis RNAs synthesized in response to phagocytosis by human macrophages by selective capture of transcribed sequences (SCOTS), Proc Natl Acad Sci USA. 1999; 96: 11554–11559.

7. Houben EN, Nguyen L, Pieters J, Interaction of pathogenic mycobacteria with the host immune system, Current Opinion in Microbiology. 2006; 9: 76-85.

8. Kimerling ME, Kluge H, Vezhnina N, Iacovazzi T, Demeulenaere T, Portaels F, Inadequacy of the current WHO re-treatment regimen in a central Siberian prison: treatment failure and MDR-TB. 1999, Int J Tuberc Lung Dis. 1999; 3: 451-453.

9. Arnoult KE, Lounis N, Guillemont J, Andries K, The challenge of new drug discovery for tuberculosis, Nature. 2011; 469: 483-490.

10. Pieters J, Mycobacterium tuberculosis and the macrophage: maintaining a balance, Cell Host Microbe. 2008; 3; 399–407.

11. Thomas JP, Baughn CO, Wilkinson RG, Shepherd RG, A new synthetic compound with antituberculous activity in mice: ethambutol (dextro-2,2'-(ethylenediimino)-di-l-butanol), Am. Rev. Respir. Dis. 1961; 83; 891-893.

12. Trauner A, Borrell S, Reither K, Gagneux S. Evolution of Drug Resistance in Tuberculosis: Recent Progress and Implications for Diagnosis and Therapy, Drugs. 2014; 74: 1063–1072.

13. Vergne I, Fratti RA, Hill PJ, Chua J, Belisle J, Deretic V, Mycobacterium tuberculosis phagosome maturation arrest: mycobacterial phosphatidylinositol analog phosphatidylinositol mannoside stimulates early endosomal fusion, Mol. Biol. Cell. 15; 751–760.

14. Villemagne B, Crauste C, Flipo M, Baulard AR, Deprez B, Willand N, Tuberculosis: The drug development pipeline at a glance, Eur J Med Chem. 2012; 51: 1-16.

15. Zahrt TC, Deretic v, Reactive nitrogen and oxygen intermediates and bacterial defenses: unusual adaptations in Mycobacterium tuberculosis, Antioxid. Redox Signal. 2002; 4: 141–159.