Tuberculosis: prevention and treatment

Tuberculosis (TB) is an airborne infectious disease which is caused by strains of mycobacteria, mainly Mycobacterium tuberculosis1. There are roughly one third of the world's population are infected with tuberculosis where nine millions of new cases reported annually2. Although tuberculosis can be prevented and treated, it continues to cause millions of deaths every year2. When infected individual coughs, sneezes or spits, M. tuberculosis is propelled into the air and infected those who breathed in the bacteria that existed in droplets of saliva3. Primarily, tuberculosis will affect the lungs, known as pulmonary tuberculosis3. It will also affect other parts of body, for instance lymph nodes, bones, brain and kidneys3. Once a person is infected with tuberculosis, there are basically three possible ways may occur. Firstly, the immune system plays a vital role and strong enough to kill the bacteria3. Secondly, immune system is not strong enough to fight off the bacteria but is able to build a defensive barrier against the bacteria3. Individuals who are latently infected with M. tuberculosis show asymptomatic where these bacteria lie dormant in the lungs and able to reactivate after years1. The disease is often reactivated in those who are immunocompromised or generally weakened. Lastly, the immune system fails to kill bacteria causing the bacteria to grow and spread towards other parts of body which is called active tuberculosis3.

In the fight of tuberculosis, World Health Organisation (WHO) recommends universal Bacille Calmette-Guérin (BCG) vaccination in the countries with high TB burdens4. BCG vaccine contains weakened form of M. tuberculosis which will induce human antibodies to fight against this type of bacteria. The efficacy of BCG vaccination can be ranging from 0% to 84%5. This may be

due to the frequency of TB exposure and quality of vaccine used, leading to arguments on BCG vaccination efficacies4. One of the greatest arguments is that BCG vaccination causing positive reactions to tuberculin skin testing and hence interfere with the diagnosis of latent TB4. Existence of evidences showing the rates of efficacy also depends on geographical location, age at vaccination and form of TB further complicate the situation. Currently, TB chemotherapy is made up of combination of a list of first-line drugs isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (EMB) 6. If the treatment fails due to bacterial drug resistance, or patient unable to tolerate, second-line drugs for instance para-aminosalicylate (PAS), fluoroquinolones, ethionamide and cycloserine are introduced6. These are considered as second line drugs generally either less potent with larger doses regimen or more toxic with serious side effects6.

Tuberculosis is presently treated in two phases, namely initial phase and continuous phase7. In initial phase, the patient will be treated with concurrent use of four first line drugs, with the aim to eradicate or control bacteria population to replicate in rapid motion and also avoid the emergence of bacteria resistance7. The treatment choices available for initial treatment include isoniazid, rifampicin, pyrazinamide and ethambutol7. Streptomycin is used rarely but can be used in patients who infected with bacteria that are resistant to isoniazid before the therapy is commenced7. The duration for initial phase is 2 months whereas the continuous phase takes 4 months7. During the four months of continuous phase, patients are treated with isoniazid and rifampicin at same doses7. Most of the TB treatment is supervised where drug administration needs to

be fully supervised by healthcare professions since lengthy duration of treatment causing incompliance in patients7. These patients who are unlikely to be compliance will be given the drugs three times a week until the course is completed while patients who able to comply with the treatment will not be supervised7.

Despite the chemotherapy treatment and BCG vaccine, TB remains as a significant infectious disease due to increasing emergence of drug resistant TB and co-infection with Human Immunodeficiency Virus (HIV) 6. Since the host defense in HIV patients is suppressed, they are more susceptible to TB infections. Moreover, drug- drug interactions between antiviral therapy and anti-TB also causing complications in treating co-infected patients6. Drug resistant TB has evolved mainly because of improper treatment or incompliance in patients who stop taking their medications before the bacteria is being fully eradicated since the duration of treatment is lengthy which takes 6-9 months8, 9. The mechanism involved includes chromosomal mutations in genes that responsible for drug targets encoding9. When there is a sequential accumulation of mutations, multi-drug resistant tuberculosis (MDR-TB) emerges where the M. tuberculosis strains will resistant to two of the most commonly used drugs, Isoniazid and Rifampicin9. Patients with MDR-TB are then relying on the second-line drug classes, fluoroquinolones and the three injectable agents namely amikacin, capreomycin, and kanamycin10, 11. The chances to cure would dramatically be reduced for patients who infected with extensively drug-resistant tuberculosis (XDR-TB), a situation where the isolated strains are resistant against any one of fluoroquinolones and at least one of three injectable drugs6.

In order to combat with the MDR-TB or XDR-TB and optimize the tuberculosis drug regimen, it is crucial to understand the mechanism of action of current using first-line drugs and how resistance is developed against these drugs.

Isoniazid (INH) or isonicotinic acid hydrazide is discovered in 1952, a bactericidal agent which active against organism of the genus Mycobacterium, especially M. tuberculosis, M. bovis and M. kansassi6, 12. In vivo, INH has shown to be bactericidal in culture over the first 48 hours which become bacteriostatic after this particular time frame12. This indicates that INH is bacteriostatic for slow replicating bacilli but is bactericidal against rapidly dividing mycobacterium. The minimal tuberculostatic concentration is 0. 025 to 0. 05ug/ml13. INH is a prodrug that needs to be activated by catalaseperoxide hemoporotein, KatG before acts by inhibiting mycolic acid synthesis and cell wall disruption in susceptible mycobacterium13, 14. This inhibitory action is only targeted to mycobacteria since other bacteria do not contain mycolic acid in the cell wall13. INH acts by inhibit enoyl acyl carrier protein (ACP) reductase, InhA, and a beta-ketoacyl-ACP synthase, KasA that are crucial in fatty acid synthesis system for mycolic acid15. Resistance to INH is believed due to mutations in gene encoding catalaseperoxidase katG or InhA or lacking KatG 9, 14. Isoniazid is metabolised in the liver, mainly by acetylation and dehydrazination where slow acetylator may experience higher concentration leads to potential toxicity before excreted in the urine within 24 hours13.

Rifampicin (RIF), discovered in 1963, is a lipophilic semisynthetic derivative of rifamycin antibiotic which is produced by the fermentation of a strain of Amycolatopsis mediterranei6, 9, 16. RIF has bactericidal activities against a

broad spectrum of microorganisms including gram-positive and gram-negative. RIF will inhibit the action of DNA-dependent RNA polymerase of mycobacteria that is encoded by rpoB through formation of a stable drug-enzyme complex9. This will suppress the initiation chain formation in RNA synthesis and hence prohibit protein synthesis in M. tuberculosis9. Development of resistance to RIF is mostly due to mutation in 81 base pair region of rpoB gene thus facilitate a straightforward approach to detect MDR-TB since 85-90% RIF-resistant strains are also resistant to INH9. RIF produces peak plasma concentration of 7ug/mL in 2 to 4 hours after ingestion of 600mg17. It also distributed well to most of the body tissues and fluids, including cerebrospinal fluid since it is lipophilic17. Following absorption from the gastrointestinal tract, RIF is eliminated rapidly in the bile with fewer amounts excreted through urine17.

Pyrazinamde (PZA) is discovered in 1954 and it produces excellent sterility effects against semidormant tubercle bacilli at slightly acidic pH6, 9. The antimicrobial activity of PZA is through interference with mycolic acid synthesis in M. tuberculosis by pyrazinoic acid, an active moiety of PZA9. Conversion of PZA to pyrazinoic acid is mediated by pyrazinamidase enzyme that is encoded by pncA gene in M. tuberculosis, thus indicating that these bacilli are sensitive to PZA9. Resistance against PZA evolved when mutation occur at pncA gene that is responsible for pyrazinamidase, hence affecting the activity of this enzyme9. PZA is well absorbed from gastrointestinal tract and is widely distributed to most tissues and fluid too17. The oral administration of 500 mg PZA produces plasma concentrations of 9-12ug/ml

after two hours and 7ug/ml after 8 hours17. PZA is metabolized in liver whereas the metabolites are excreted through renal glomerular filtration17.

Ethambutol (EMB) is discovered in 1962, acts as bacteriostatic agent and is active against undergoing cell division6, 18. EMB primarily targets on impairment of cell wall polymerization by inhibits arabinosy transferase, a vital enzyme responsible for mycobacteria cell wall biosynthesis9, 18. Since arabinosy transferase enzyme is encoded by embC-embA-embB genes, resistance against EMB evolved is believed due to mutation of these genes9. EMB is currently used as one of the first-line treatment for tuberculosis mainly because of its synergistic effect with other front-line drugs and its low toxicity property18. There is roughly 75-80% of an oral dose of EMB is rapidly absorbed in gastrointestinal tract with absorption unaffected when administered with foods19. In addition, EMB is distributed widely to body tissues and fluid, including cerebrospinal fluid before being metabolized in the liver and excreted in urine19.

Streptomycin (SM) is an aminoglycoside antibiotic, used as first line treatment for TB when it first discovered in 19441, 6. Streptomycin is isolated from the bacteria Streptomyces griseus and its antimicrobial effects against M. tuberculosis is highly effective when use in combination with other first line agents20. However, SM is no longer considered as first line treatment as resistance against it has developed rapidly1. The optimum pH for SM is at pH8 where its bacteriostatic activity will reduce with increasingly acidic environment20. SM acts by binding tightly to A site of 16S ribosomal RNA subunit, interferes with mRNA translation, causing faulty protein being produced1, 9. Resistant emergence when the mutation occurs at gene rpsL

and rrs that encoded for 16S and S12 ribosomal protein1, 9. Upon administration, SM is poorly absorbed from gastrointestinal tract and mostly administered parentally1. SM is mostly excreted in urine and patients with low renal profile might experience toxicity such as neurotoxic reactions1.

When the first line treatment is no longer suitable for patients or patients develop multi-drug resistance TB, second line drugs will then be introduced in combating the TB. Second line drugs that are mostly used include Ethionamide (ETH), Cycloserine (CS), Para-Aminosalicylic Acid (PAS) and Fluoroquinolones (FQ).

ETH has been in use since 1960s, is a structural analogue of INH and it targets at inhibition of mycolic acid biosynthesis in tubercle bacilli9, 21. INH however is much more potent than ETH since the minimal inhibitory concentration for ETH is 0. 5-5. 0ug/mL21. Resistance evolved due to mutation at gene InhA and ethA which encode for oxygenase enzyme in activation of ETH 9.

In vitro, CS has inhibitory effect on M. tuberculosis at 5-200ug/mL and there is no cross resistance occurred between CS and other drugs13. CS acts by interfereing the biosynthesis of bacterial cell wall13. CS is well absorbed in gastrointestinal tract and also widely distributed to body tissues and fluid including cerebrospinal fluid13.

PAS was first introduced as first line drug but being replaced by Ethambutol in 1960s1. It acts bacteriostatically with possessing inhibitory effect at concentration less than 1mg/ml by interfere with folic acid metabolism in bacteria1. PAS is readily absorbed from gastrointestinal tract and distributed

well throughout the body. Approximately 80% of the drugs will be excreted via kidney after being metabolized to acetylated form1.

Moxifloxacin and Gatifloxacin are both been synthesized and evaluated as excellent bactericidal agents through inhibiting DNA gyrase, an ATP-dependent enzymes topoisomerase II which is responsible in bacteria DNA transcription9. DNA gyrase is consisted of two subunits that is arranged in a complex, is encoded by two different genes, gyrA and gyrB where mutations at gyrA will normally cause bacteria resistance to these new generation of flouroquinolones9.

Due to the increasing incidence of multidrug resistance TB, it is highly desirable to develop new drugs that are not only potent and effective against current resistant strains of M. tuberculosis but also possess shorter treatment duration since most of the incompliance of patients is brought up by lengthy TB treatment. Most of the mechanisms of action of current treatments are involved in interfering the bacterial DNA synthesis, protein and mycolic cell wall biosynthesis. The enzymes that participate in these pathways could also be the target of newly designed drugs such as TMC207, one of the new drugs which are currently under investigations and clinical trials.

TMC207 is a member of diarylquinoline class of compound which target at adenosine triphosphate (ATP) synthase by binding to subunit C of the synthase, blocking the energy pathway of mycobacteria22, 23. In vitro, TMC207 not only possesses ability to inhibit both drug sensitive and resistant M. tuberculosis isolates, but also able to sterilize the patient through killing

the dormant bacilli bactericidally22. TMC207 showed a minimum inhibitory concentration of 0. 03ug/mL against M. tuberculosis, suggesting a more potent agent compared to current first- line treatments such as isoniazid and rifampicin23. Apart from that, its synergistic effect with pyrazinamide could promise as effective drug combination for sterilizing the patients against TB22. A phase I clinical trials which involved short terms administration of TMC207 in healthy individuals showing no adverse effects and the subjects are well tolerated with it23. However, it is essential to investigate the selectivity of TMC207 against mammalian ATP synthase with longer periods to ensure the patients' safety when administered with TMC207.

Thiacetazone (TAC) is widely used as second line anti-TB agent against multiresistant tuberculosis at present24. TAC acts by interferes the biosynthesis pathway of mycolic acid in tubercle bacilli24. The fact that M. tuberculosis has been difficult to eradicate and remains persistent is due to its cell wall that composed of mycolic acid which is resistant against chemical injury, dehydration and also has low permeability to antibiotics24. Mycolic acid contains cyclopropane rings that is activated through cyclopropane mycolic acid synthase (CMASs), has a significant contribution to tuberculosis24. By inhibiting the cyclopropanation, the cell wall biosynthesis will then be interrupted, introducing the bactericidal effects24.

The aim of this research is to synthesis and evaluates the analogues of Thiacetazone which might be potential anti tuberculosis agents. The analogues will be tested against different strains of mycobacteria in lab. The target actions of these analogues will also be identified based on the structure of the analogues.

The above analogue is synthesized when a benzylaldehyde reacts with a primary amine. This is a condensation process and an imine is produced. The changes at position R1 to R3 with different electron withdrawing groups are first planned to be evaluated. However, the plan is prohibited since the corresponding structures are either unavailable or too expensive that falling outside the budget. After revised on the previous analogues that were discovered and their respective MIC values obtained from lab, the structures of new analogues that are going to be evaluated are finally sorted out. The R1 to R3 positions would be replaced by either a -chloro or a -methoxy with R8 position would either be an amine, a methyl or a benzene ring. A chloro is used at position R1 to R3 since it is electron withdrawing, big and lipophilic molecule whereas the methoxy group is electron donating, small and quite lipophilic. For R8 position, an amine is selected because it is electron withdrawing and small. A methyl is also selected since it is quite lipophilic, small and electron donating. On the other hand, benzene ring which is highly lipophilic, neither electron donating nor withdrawing group might have a different effect on the analogue synthesized.

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Mycolic acids are a complex mixture of branched, long-chain fatty acids, representing key components of the highly hydrophobic mycobacterial cell wall. Pathogenic mycobacteria carry mycolic acid sub-types that contain cyclopropane rings. Double bonds at specific sites on mycolic acid precursors are modified by the action of cyclopropane mycolic acid synthases (CMASs). The latter belong to a family of S-adenosyl-methionine-dependent methyl transferases, of which several have been well studied in Mycobacterium tuberculosis, namely, MmaA1 through A4, PcaA and CmaA2. Cyclopropanated mycolic acids are key factors participating in cell envelope permeability, host immunomodulation and persistence of M. tuberculosis. While several antitubercular agents inhibit mycolic acid synthesis, to date, the CMASs have not been shown to be drug targets.

We have employed various complementary approaches to show that the antitubercular drug, thiacetazone (TAC), and its chemical analogues, inhibit mycolic acid cyclopropanation. Dramatic changes in the content and ratio of mycolic acids in the vaccine strain Mycobacterium bovis BCG, as well as in the related pathogenic species Mycobacterium marinum were observed after treatment with the drugs. Combination of thin layer chromatography, mass

spectrometry and Nuclear Magnetic Resonance (NMR) analyses of mycolic acids purified from drug-treated mycobacteria showed a significant loss of cyclopropanation in both the ?- and oxygenated mycolate sub-types. Additionally, High-Resolution Magic Angle Spinning (HR-MAS) NMR analyses on whole cells was used to detect cell wall-associated mycolates and to quantify the cyclopropanation status of the cell envelope. Further, overexpression of cmaA2, mmaA2 or pcaA in mycobacteria partially reversed the effects of TAC and its analogue on mycolic acid cyclopropanation, suggesting that the drugs act directly on CMASs.

This is a first report on the mechanism of action of TAC, demonstrating the CMASs as its cellular targets in mycobacteria. The implications of this study may be important for the design of alternative strategies for tuberculosis treatment.