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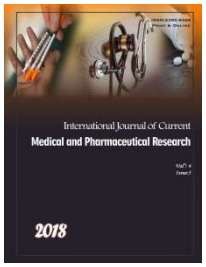

NON ANTIBIOTIC THERAPY FOR MYCOBACTERIUM TUBERCULOSIS INFECTION

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ABSTRACT

Despite many recent developments in the management of tuberculosis [TB], it still remains a world-wide menace due to its prolonged duration and high cost of therapy, marked drug toxicity, failure of the patient to complete the prescribed pharmacotherapy. Defense mechanisms of the organism against host immunity and the emergence of multi drug resistant strains [MDR] of mycobacterium tuberculosis [Mtb] adds to the difficulty of the treatment. Newly recommended antibiotics for resistant forms of tuberculosis are Linezolid, Bedaquiline and Delamalid. These drugs have their own potential hematological and neurological toxicities and are expensive. Hence there is a need to find out newer antibiotics or to use the antimicrobial properties of non antibiotic drugs to treat the resistant forms of TB. Drugs which are not antibiotics but have been proved to possess anti tubercular activity are -1] Phenothiazines like Chlorpromazine and Thioridazine 2] proton pump inhibitor like Lansoprazole 3] Antihypertensive like L type calcium channel blocker Verapamil and 4] Anti diabetic like biguanide derivative Metformin. They inhibit bacterial efflux pumps and also affect the energy metabolism of the organism. In addition they modify the host defense mechanisms like increased mitochondrial ROS production and enhanced fusion of lysosome with phagosome of the macrophages. These drugs are less expensive than the newer drugs recommended by WHO. Hence there is a need for extensive clinical trials of these non antibiotic drugs to establish their use as an adjunct or as first or second line of treatment in the management of Mtb.

INTRODUCTION

Tuberculosis [TB] is still a world-wide menace which is getting more serious with the immune compromised conditions like HIV infection. Despite many recent developments in the management of tuberculosis, it still remains unsatisfactory due to its prolonged duration and high cost of therapy, high drug toxicity, failure of the patient to complete the recommended pharmacotherapy, defense mechanisms of the organism against host immunity and the most important the emergence of multi drug resistant strains [MDR] of mycobacterium tuberculosis [Mtb]. These bacteria have impermeable mycolic acid cell wall which favors their longer survival in the host cells. Their long duplication time also adds to the development of drug resistance to anti TB drugs. This demands an urgency for discovering newer effective drugs to treat this infection.[1,2]

Mechanism of resistance of Mtb organism includes 1].Various defense mechanisms of Mtb- cell wall virulence factor like mycolic acid in the cell wall, inhibition of phagosome-lysosome fusion of macrophages, inhibition of phagosomal acidification and protection against oxidative radicals produced by macrophages[3] 2]. Efflux of anti TB drugs by the mycobacterial efflux pumps [4-7] and 3] the development of mutations at the gene coding level of the antibiotic target.[8] The initial response of infected organism to the noxious stimuli and the antibiotic is to over express the efflux pumps. [9-12]

Resistance produced by Mtb organisms against the available anti TB drugs are of various types. Infections resistant to first line anti TB drugs like Isoniazide and Rifampicin are termed as multidrug resistant tuberculosis[13] Resistance to second line drugs like Amikacin, Kanamycin, Capreomycin and Fluoroquinolones in addition to Isoniazide and Rifampicin is termed as extensively drug resistant tuberculosis [XDR TB].[14]Recently resistance to all the anti TB drugs have been noted in Italy, Iran, South Africa and also in India which is termed as totally drug resistant tuberculosis [TDR TB]. [15] Newly recommended antibiotics for resistant forms of tuberculosis are Linezolid, Bedaquiline and Delamalid. These

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drugs have their own potential hematological and neurological toxicities. WHO still continues to recommend the use of these extremely costly drugs for the treatment of MDR and XDR TB.[16-19]Now there is a need to find out newer antibiotics or to use the antimicrobial actions of non antibiotic drugs to treat the resistant forms of TB.

Drugs which are not antibiotics but have been proved to possess the anti TB activity are -1] Phenothiazines like Chlorpromazine and Thioridazine.[20] 2] Proton pump inhibitor like Lansoprazole.[21] 3]Antihypertensive like L type calcium channel blocker Verapamil[22] and 4] Anti diabetic like biguanide derivative Metformin.[23]

Phenothiazines like Chlorpromazine [CPZ] and Thioridazine [TZ] are heterocyclic compounds. In vitro anti mycobacterial property of CPZ was observed in the year 1977[24] which was eventually confirmed after 10 years.[20] CPZ has its own serious adverse drug reactions.[25] TZ was found to have fewer side effects as compared to CPZ, when it was used with proper doses and precautions along with frequent evaluation of the patient for cardiomyopathy. TZ has been used for more than 60years and is found to be safe. It was tested for in vitro activity against the both susceptible and resistant strains of Mtb and was found to be equally effective.[26,27]

Both CPZ and TZ are active against susceptible and resistant strains [MDR and XDR] of Mtb.[28-31]CPZ reduces the resistance by acting on the cell wall of Mtb. [32,33] Similarly TZ was also found to reduce the resistance to the first line anti TB drugs at the dose which is used clinically to treat psychosis.[34] The role of efflux pumps in the organism which pumps out the antibiotics was confirmed in the MDR TB.[5,35] Studies have shown that TZ reversed the resistance of Mtb for INH through the interference in the over expression of genes of efflux pumps of organisms mmpL7,p55,efpA,mmr,Rv1258c and Rv2459. [4]

Besides the interference in efflux pumps of organisms, the other possible mechanisms of TZ against Mtb are the disturbances in the genes that code for essential proteins of cell envelope[36] and those that code for proteins of plasma membrane and are involved in governing the essential energy production, active transport and permeability in response to oxidative stress and antibiotics.[37] The affected genes were those that encode efflux pumps which extrude antibiotics and also the genes that encode for oxido reductases, enzymes of fatty acid metabolism and aerobic respiration.[38]

It was observed in several studies that TZ acts on the components of mycobacterial respiratory chain namely type II NADH menaquinone oxidoreductase [NDH2] which involves in the ATP oxidative phosphorylation.NDH 2 catalyses the first reaction of the electron transfer chain of Mtb that leads to ATP oxidative phosphorylation. This forms the molecular target for TZ against Mtb. Modification in the respiration of Mtb with defective ATP synthesis and NADH regeneration results in to hypoxic non replicating mycobacteria. This mechanism of TZ is responsible to reduce the population of both actively replicating and dormant Mtb.[39-41]

CPZ and TZ were found to be effective against the drug sensitive and MDR strains of Mtb and also against XDR strains.[42] TZ showed synergistic effect with the first line anti TB drugs.[43] TZ gets concentrated in the phagolysosome and it was found to enhance the killing of intracellular Mtb.[44,45] The accelerated fusion of phagosome with the lysosome of the macrophage, inhibition of ion channels and efflux of ions from phagolysosomal unit, acidification of phagolysosomal unit and activation of hydrolytic enzymes through the coupling of the vesicular ATPases of phagolysosome favour bacterial killing.[46] TZ inhibits bacterial efflux pumps.[4,5,35] The inhibition of the efflux of ions from phagolysosomal unit of macrophage leads to significant increase in intracellular stores of Ca2+ resulting in to indirect acidification of the compartment and the activation of hydrolytic enzymes via coupling of vesicular ATPases and killing of entrapped Mtb.[4,43]

The studies supported the use of non antibiotic drug TZ against resistant Mtb infection used either in combination with other anti TB drugs or as an alternative therapeutic drug alone to treat Mtb.[47-51] When TZ was used in a group of patients of XDR TB as a mono therapy or in combination with anti TB drugs, it resulted in to the complete cure.[52] Udwadia *et al*[53] and Abbate *et al*[52] have observed that the use of TZ was safe without having any significant effect on QT interval and on other cardiac properties.

Before starting treatment with TZ, patients should be evaluated for possible cardiomyopathy. The drug is safe up to 1000mgs/day. TZ needs to be introduced to the patient starting with the dose of 25mg/day and gradually increasing weekly up to 50,100 and 200mg per day. This protocol has been proved to be safe as it did not affect the QT interval. [52-55] Those patients are the good candidates for the treatment with TZ as a single drug or as an adjunct drug to anti TB drugs in whom resistance to antibiotic is produced possibly due to over expression of efflux pump system. At present there are few laboratories which have the facilities to assay the efflux pump status of the infecting Mtb in the given sample which helps to confirm the ability of TZ to reverse the resistance.[56]

TZ is quite safe as compared to the side effects noted in patients of MDR TB treated with the newly approved anti TB drugs like Linezolid, Fluoroquinilone, Bedaquiline or Delamalid. These drugs are also expensive than TZ. As compared to all other anti TB drugs TZ is cost effective.[57] Though safety profile of TZ is good, cardiac evaluation is needed before starting the drug as certain class of population like eastern Europeans have mutations in CYP 450 that may lead to raised TZ levels and subsequent cardiac toxicity.[54,55] WHO has recommended Linezolid to be included in the regimens of anti TB drugs used for MDR TB and XDR TB infection. [16,58] But Linezolid has very narrow therapeutic window and is associated with the significant toxicities like hematological disorders and neuropathy.[18,59,60]

In comparison TZ is effective in a dose of 200 mg per day[52] in combination with the first line anti TB drugs for any form of TB. TZ gets concentrated by phagolysosome and is effective against bacterial efflux pumps responsible for MDR TB and thus enhances the bacterial killing.

Extensive clinical trials are needed to study the potential of TZ as an anti TB drug so that this drug can be considered for regular use to treat tuberculosis as it is quite economical and free from adverse reaction when used with proper precautions and in proper dose in comparison with other available ant TB drugs at present.

Proton pump inhibitor-Lansoprazole

Proton pump inhibitors [PPIs] are weak bases and consist of two moieties -1]substituted pyridine and 2] benzimidazole.

These are acid activated pro drugs which get converted in to tetracyclic sulfenamide. Activated sulfenamide binds to the parietal cell H+K+ATPase [proton pumps] and inhibits acid release.[61-63] PPIs are indicated for acid peptic disorders, NSAID induced gastric mucosal injury, Zollinger Ellison syndrome and H.Pylori infection.[64] To treat H. Pylori infection Lansoprazole [LPZ] is preferred due to its specific structure activity relationship like the presence of trifluoroethoxy group at C 4 position of pyridine ring in its structure which makes it more potent antibacterial agent than the other PPIs.[65]

LPZ was found to have intracellular anti tubercular activity. By sulfoxide reduction pro drug LPZ gets intra cellularly converted in to a highly stable metabolite LPZ sulfide [LPZS] which has selective antimicrobial action against Mtb. LPZS reduces the synthesis of bacterial cellular ATP due to the inhibition of cellular respiration.[64] LPZS prevents ATP generation from ADP by acting on cytochrome bc 1 complex [complex III] which is an essential respiratory chain component for ATP synthesis. The ratio of ADP/ATP in mycobacterium treated with LPZS was about 7 fold more than in untreated group.[66] Cytochrome b subunit of cytochrome bc 1 complex-Qcr B- was identified as drug target for Mtb which is an integral member of bc 1 complex of respiratory electron transport chain.[67,68] LPZS is a novel class of QcrB inhibitor which is emerging as a highly promising drug against Mtb. LPZS is a highly safe and a promising anti TB lead compound which does not inhibit gastric proton pumps. The differential activation of pro drug LPZ in gastric parietal cells [sulfenic acid and sulfenamide intermediates] and in the mycobacterial cell [sufoxide reduction of LPZ to LPZS] makes the LPZS a highly effective anti TB drug. Thus targeting of cytochrome bc 1 complex-QcrB of Mtb forms the new mechanism of action of anti TB drug.

Antihypertensive -Verapamil

L type calcium channel blocker verapamil has been tried for its activity against Mtb. Verapamil was tested against drug resistant Mtb both in vitro and in the human macrophages. This ion channel blocker drug inhibits drug efflux from bacteria and exhibits synergistic inhibitory activity against Mtb when combined with anti TB drugs like Isoniazide and Rifampicin.[69,70] Whenever Mtb is exposed to antibiotic, it's efflux genes and related efflux pumps get over expressed within Mtb. The inhibition of this efflux pump activity by verapamil increases the intra bacterial concentration of the drug which helps in the bacterial killing and overcoming the drug resistance.[71-74] This also prevents the development of drug resistant mutants. Verapamil by inhibiting efflux pumps acts as a promising anti tubercular drug.[73-76]

Verapamil showed highly rapid anti tubercular activity due to the interference in the energy metabolism of the Mtb resulting in to decreased intracellular ATP synthesis. Key electron transport chain enzymes required for energy production in the bacteria are thought to be inhibited by verapamil.[77,78] This decreases the energy supply required for bacterial efflux pumps and reduces the drug resistance. Thus verapamil has anti TB activity and reduces the drug resistance.[79]

Verapamil also promotes the acidification in the phagosome vacuoles essential for microbial killing and their digestion through lysosomal hydrolytic enzymes.[80,81] Inhibition of bacterial vacuolar efflux pumps and increased concentration of verapamil in the bacteria enhances the transcription of proton pump V-ATPases resulting in to increased phagosomal acidification[82] and increase in Ca2+ concentration in the lumen. This results in to the activation of acidification dependant lysosomal hydrolases and enhances macrophage mediated mycobacterial killing.[83]

Verapamil induced enhanced killing activity of macrophages depends upon the availability of intracellular K+ and Ca2+. Voltage gated calcium channels negatively regulate protective immunity to Mtb.[84-86] Gupta *et al* demonstrated that the increased intracellular Ca2+ stores in the macrophages resulted in to reduced Mtb population as a result of improved host macrophage immunty.[84]

Thus verapamil targets mycobacteria and host macrophages resulting in to the inhibition of respiratory chain complexes and energy production required for bacterial efflux activity. This also promotes phagosomal acidification, enhances transcription of hydrolases in host cells and increases intra macrophage Ca^{2+} concentration resulting in to the inhibition of bacterial growth which contributes for its synergistic activity with anti TB drugs. Overall verapamil is well tolerated with fewer side effects.

Antidiabetic- Metformin

Evasion of either innate or adoptive host cell immune responses by the organisms favors the prolongation of infection.[87-89]

The major host innate immune responses against microbia include the generation of reactive oxygen species [ROS],the phagosomal activity enhancing phagosome-lysosome fusion and the autophagy pathway which destroys the intracellular pathogens.[90,91] Adenosine monophosphate activated protein kinase [AMPK] plays an important role in the regulation of host immunity. Disturbances in the phagosomal activity, autophagy pathway and AMPK activation are responsible for virulence of Mtb organisms.[92,93]

AMPK activating anti diabetic drug metformin was found to inhibit the growth of intracellular Mtb and attenuate the disease related immune pathology. Amit Singh *et al* found that treatment with metformin attenuated the growth of M.bovis bacillus calmette Guerin in the human cell line and also in the H37Rv strain of Mtb. It also restricted the replication of intracellular MDR strain of Mtb. AMPK activation by metformin was responsible for its anti TB activity. Metformin selectively induced mitochondrial ROS [mROS] production.This could be due to the inhibition of mitochondrial complex-1[NADH dehydrogenase] activation which resulted in to the accelerated phagosome-lysosome fusion.[23,94,95] Production of mROS triggers the intrinsic apoptotic pathway leading to cell death as a result of the dissipation of mitochondrial membrane potential and intra cytoplasmic release of cytochrome C.[96]

Studies showed that metformin enhanced the efficacy of the conventional anti TB drugs and decreased the tissue bacillary load in the mice lung. After treatment with metformin, tissue related pathology of tuberculosis in the lung and spleen was found to resolve associated with accelerated bacillary clearance.[97]

Metformin treatment was also found to improve host immune responses as observed by increased CD 4 and CD 8 cell count in the lungs of mice infected with Mtb and increased

mycobacteria specific interferon secreting CD8 cells which contributed to the control of Mtb infection.[23]

AMPK is a negative regulator of inflammation whose activation by metformin imparts anti inflammatory effect.[98] The inflammatory responses in the infected animals were reduced by metformin as it normalized various inflammatory pathways and also reduced the expression of related inflammatory genes such as IL-1B,IL-6,TNF alpha, CXCL-5 CXCL-10 and MCP-1 in the mouse lung. [23]

Reduction in the severity of tubercular infection, better clinical outcome and reduced mortality in the patients of diabetes mellitus with tuberculosis was observed when they received antiTB drugs along with metformin than with other anti diabetic drugs.[99] Metformin reduced the incidence of latent tuberculosis in the diabetic patients as confirmed by T- SPOT test reactivity.[23]

Metformin when combined with conventional anti TB drugs in the mice infected with Mtb reduced the severity of bacillary load in the lungs. In this animal study dose of metformin used was 500mg/kg which was equivalent to 2430mg/day for 60kg human beings, which is lesser than maximum permissible human dose of 3gms/day. [23]

These observations related to metformin as an adjunct drug to treat tuberculosis opens the new promising avenue for host directed anti TB therapy which can be used for improved clinical outcome in TB patients.

CONCLUSION

These non antibiotic drugs inhibit the bacterial efflux pumps and also affect their energy metabolism and reduce ATP synthesis. They improve the host defense mechanisms like mROS production and acceleration of phagosome lysosome fusion in macrophages . Thus they act as anti TB drugs. Hence there is need for extensive clinical trials of these drugs to establish their use in the of treatment of Mtb either as an adjunct or as first line or second line drugs.

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