

Review



'PYRAZINAMIDE A STERILIZING DRUG AGAINST MYCOBACTERIUM TUBERCULOSIS -A REVIEW

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Submitted on 14.08.16;

Revised on: 24.08.16;

Accepted on: 31.08.16

ABSTRACT

Compared to the treatment of other infectious diseases, the treatment time for Tuberculosis is long, which makes patient compliance very difficult. The main reason for this lengthy treatment is to prevent relapse of Tuberculosis. Antibiotics are usually active against growing bacteria but are less effective against non growing bacteria. Current Antitubercular drugs such as isoniazid are mainly active against growing bacteria and are not effective against the persisters and dormant bacteria. Pyrazinamide is an important first-line Antitubercular drug used in combination with other antitubercular drugs for the treatment of both drug-susceptible Tuberculosis and multidrug-resistant tuberculosis (MDR-TB). Its high activity against persister bacteria is responsible for Pyrazinamide unique sterilizing activity, which shortens the Tuberculosis treatment period from 9-12 months to 6 months. Because of its indispensable sterilizing activity, all new TB regimens in clinical development include Pyrazinamide. Aspartate decarboxylase (PanD) is a new target involved in Pyrazinamide action. Recently, identified a new gene PanD encoding aspartate decarboxylase, involved in the synthesis of Panthothenate (vit B₅) which in turn required for the synthesis of CoA, a molecule that is at the center of all energy metabolism and allows carbohydrates, fats and proteins to be burned as energy sources. These findings shed new light on the mode of action of Pyrazinamide and may help in the design of new drugs that shorten therapy.

KEYWORDS:- Pyrazinamide, Tuberculosis, Multi Drug Résistance Tuberculosis, Pyrazinoic Acid

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Indian Research Journal of Pharmacy and Science; 10(2016) 773-779
Journal Home Page: <https://www.irjps.in>

INTRODUCTION

Tuberculosis or TB (short for tubercle bacillus), is a widespread infectious disease caused by various strains of Mycobacterium usually Mycobacterium tuberculosis. Tuberculosis typically attacks the Lungs, but can also affect other parts of the body. It is spread through the air when people who have an active TB infection cough, sneeze or otherwise transmit respiratory fluids through the air. Most infections do not have symptoms, known as latent tuberculosis. About one in ten latent infections eventually progresses to active disease which, if left untreated, kills more than 50% of those so infected.

Two billion people, one third of the world's population, are infected with tuberculosis and Mycobacterium tuberculosis is responsible for 8.8 million new infections and 1.6 million deaths each year. The emergence of multi drug resistant M.tuberculosis strains, that are resistant against the current first line drugs like Isoniazid and Rifampin, contribute to the spread and worsen the situation by lengthening the treatment considerably from 6 months to nearly 2 years and thereby increasing the cost for therapy 20 fold.

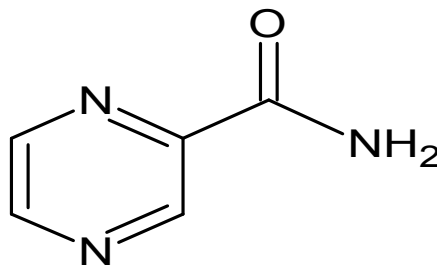
According to latest WHO report, there were an estimated 8.6 million incident cases of tuberculosis in 2012 and 1.3 million deaths were attributed to the disease. More than half of a million cases occurred in children and 320,000 deaths were reported among HIV infected persons. However disturbing is the emergence of drug resistance.

History of Mycobacterium tuberculosis is very old and it has been known since 2400BC when antiquity-fragments of the spinal column from Egyptian mummies show definite pathological

signs of tubercular decay. Exact pathological and anatomical description of the disease appeared in 17th century. Introduction of sanatorium was the first real step against TB. In 1854 Hermann Brehmer presented his doctoral dissertation bearing the auspicious title 'Tuberculosis is a curable disease'. New advances then followed in rapid succession and in 1865 a French military doctor Jean-Antoine Villemin postulated that a specific microorganism is the cause of the disease.¹

PROBLEMS OF CURRENT TB DRUGS

Although DOTS can cure TB in most of the cases, some serious problems still remain. Among those, the TB persistence and drug resistance are most challenging. Compared to the treatment of other infectious diseases, the treatment time for TB is long, which makes patient compliance very difficult. The main reason for this lengthy treatment is to prevent relapse of TB. Antibiotics are usually active against growing bacteria but are less effective against non growing bacteria. Current TB drugs such as isoniazid are mainly active against growing bacteria and are not effective against the persisters and dormant bacteria. PZA kills non replicating persisters than other TB drugs fails to kill. The incapability of host immune system to eliminate tubercle bacilli in the lesions could be another factor that leads to the persistence of TB. The first TB chemotherapy, streptomycin, came into use to treat TB in 1943. The increase of multidrug-resistant strains resistant to these widely available and affordable anti-tubercular drugs is a growing clinical problem. Fifty million people have already been infected with drug-resistant TB.²



Pyrazinamide

PURPOSE OF WORK

The purpose of work is to review various targets of Pyrazinamide against Mycobacterium tuberculosis. PZA acts differently from common antibiotics by inhibiting multiple targets such as energy

production, trans-translation and Panthothenate coenzyme. PZA is a unique anti-tuberculosis drug that plays a key role in shortening the TB therapy. PZA kills non-replicating persisters than other TB

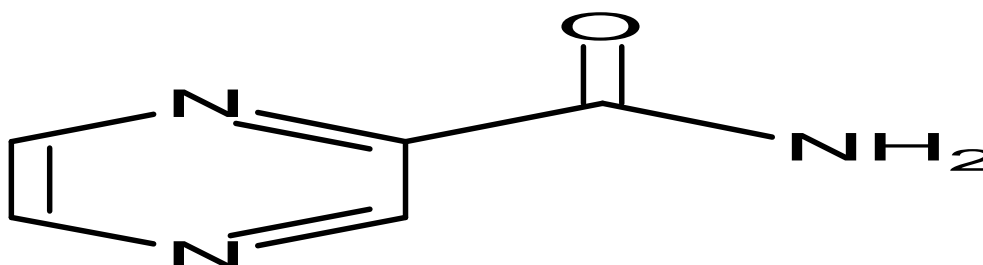
drugs fail to kill, and thus making it an essential drug for inclusion in any drug combinations for treating drug susceptible and drug-resistant TB such as MDR-TB. Finally, the story of PZA has important implications for not only TB therapy but also chemotherapy in general. PZA serves as a model prototype persister drug and hopefully a 'tipping point' that inspires new efforts at developing a new type of antibiotics or drugs that target non-replicating persisters for improved treatment of not only TB but also other persistent bacterial infections.

PZA is a unique anti-tuberculosis drug that plays a key role in shortening the Tb therapy. PZA kills non replicating persisters that other TB drugs fail to kill, and thus making it an essential drug for inclusion in any drug combinations for treating drug susceptible and drug-resistant TB such as MDR-TB. PZA acts differently from common antibiotics by inhibiting multiple targets such as energy production, trans-translation and perhaps Panthothenate/coenzyme A required for persister survival. Resistance to PZA is mostly caused by mutations in the *pncA* gene (Pyrazinamidase

encoding gene) involved in conversion of the prodrug PZA to the active form POA (Pyrazinoic acid). Mutations in the drug target RpsA (ribosomal protein S1) are also found in some PZA-resistant strains. The recent finding that *panD* (Aspartate decarboxylase) mutations are found in some PZA-resistant strains without *pncA* or RpsA mutations may suggest a third PZA resistance gene and a potential new target of PZA. Current phenotype based PZA susceptibility testing is not reliable due to false resistance, and sequencing of the *PncA* gene represents a more rapid, cost-effective and more reliable molecular test for PZA susceptibility testing and should be used for guiding improved treatment of MDR/XDR-TB. Finally, the story of PZA has important implications for not only TB therapy but also chemotherapy in general. PZA serves as a model prototype persister drug and hopefully a 'tipping point' that inspires new efforts at developing a new type of antibiotics or drugs that target non-replicating persisters for improved treatment of not only TB but also persister bacterial infections.³

STRUCTURE OF PYRAZINAMIDE

- Synthetic analogue of Nicotinamide
- More active in acidic medium
- It consist of Pyrazine nucleus



- Molecular formula: -C₅H₅N₃O
- Molecular weight :-123.113g/mole
- IUPAC name :-Pyrazine-2- carboxamide

MECHANISM OF ACTION

Pyrazinamide (PZA) has no activity in vitro under normal culture condition at neutral pH, but is active only at an acid pH (pH 5.5). PZA only kills *M.tuberculosis* slowly in vitro at acid p^H. In vivo, PZA has high sterilizing activity against persisters

in an acidic environment that is present during inflammation, which is responsible for its ability to shorten TB therapy. PZA is a prodrug that is converted to the active form POA by Pyrazinamidase encoded by the *PncA* gene in *M.tuberculosis*. PZA enters bacilli through passive diffusion and is converted into POA by the

cytoplasmic Pyrazinamidase encoded by PncA. POA then gets out of the cell through passive diffusion and a deficient efflux mechanism in M.tuberculosis.

Once POA is outside the cell, if the extracellular P^H is acidic (P^H 5.5), a small proportion of POA will become uncharged protonated acid HPOA, which readily permeates through the membrane. The acid-facilitated POA influx can overcome the weak deficient POA efflux, which causes accumulation of POA in M.tuberculosis cells at acid P^H over time. The HPOA brings protons into the cell and this could eventually cause cytoplasmic acidification such that vital enzymes could be inhibited.⁴

SIDE EFFECTS OF PYRAZINAMIDE

- Fever
- Dysuria
- Hepatotoxicity
- Nausea
- Vomiting
- Anorexia
- Arthralgia
- Hypersensitivity reactions-Urticaria, Pruritis and rashes
- Gout

- Hyperuricaemia is due to inhibition of uric acid secretion in Kidney

CONTRAINDICATIONS

Pyrazinamide is contraindicated in persons

- With Severe hepatic damage
- Who have shown hypersensitivity to it
- With acute gout

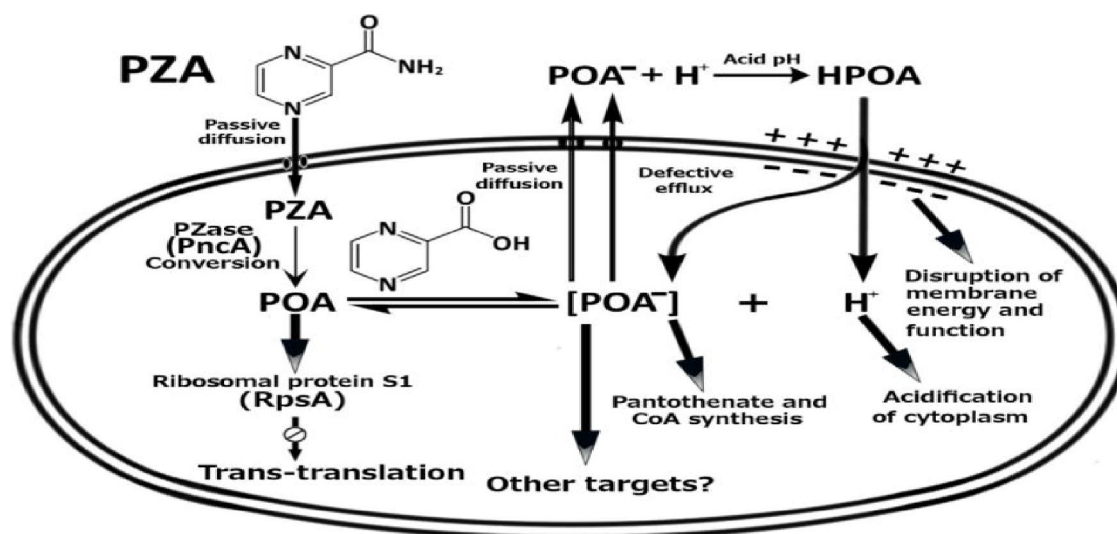
PHARMACOKINETIC DATA

- Absorption :-Rapidly absorbed from GIT
- Bioavailability :->90%
- Metabolism :-by liver
- Biological half life :-6-19hrs
- Excretion :-Renal
- Protein bound :-10%
- Route of administration :-oral
- It has good penetration in CSF because of which is highly useful in meningeal TB.

DOSE

Daily dose is now limited to 25-30mg/Kg which produces only a low incidence of hepatotoxicity.

FINDINGS AND DISCUSSION:-



This study suggests that Pyrazinamide is an important sterilizing drug that shortens tuberculosis

(TB) therapy. However, the mechanism of action of Pyrazinamide is poorly understood because of its

unusual properties. Here they show that POA, the active moiety of Pyrazinamide, disrupted membrane energetic and inhibited membrane transport function in *M.tuberculosis*. The preferential activity of Pyrazinamide against old non-replicating bacilli correlated with their low membrane potential and the disruption of membrane potential by POA and acid pH. These findings shed new light on the mode of action of PZA and may help in the design of new drugs that shorten therapy. POA inhibited the protein and RNA synthesis and serine uptake as well as

Various pyrazine derivatives and Pyrazinamide analogues, were assayed, their antimycobacterial activities in vitro in order to find new drugs which are more active against *Mycobacterium tuberculosis* than PZA. Of the drugs synthesized, four drugs namely

- ✓ Pyrazine thiocarboxamide
 - ✓ N-hydroxymethyl-Pyrazine thiocarboxamide
 - ✓ Pyrazinoic acid n-octyl ester
 - ✓ Pyrazinoic acid pivaloyloxymethyl ester,
- were not only bacteriostatic but also bactericidal against mycobacterial in vitro under conditions in which PZA showed no or little activity. In conclusion, these four drugs are possible candidates for new antimycobacterial agents.⁶

Effect of pH on antimycobacterial activity of the drug

Pyrazinamide is more effective in acidic media than in a neutral one, the growth of *Mycobacteria* is rather poor and inconsistent in acidic media. To determine which pH is appropriate for a screening medium, we compared the antimycobacterial activities of Pyrazinamide in Middlebrook 7H9 broth adjusted to pH 6 and to pH5.5. The growth of *Mycobacterial tuberculosis* in medium with a pH of 5.5 was much poorer than in medium with a pH of 6. The same results were observed with other analogs of Pyrazinamide.

Minimal effective concentrations of the drugs (MIC)

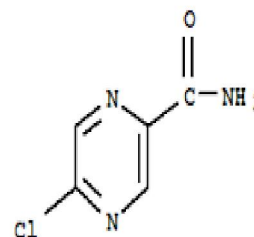
The MICs of the four drugs were determined. Under the experimental conclusion, PZA at 200mg/ml was not bactericidal against any of the *Mycobacterial species* tested. Pyrazinoic acid pivaloyloxymethyl ester at 50mg/ml was highly bactericidal against *M.tuberculosis*.

disruption of membrane potential at acid pH. The observation that uptake of uracil and Methionine was significantly reduced in the presence of POA at acid pH implies that both RNA and protein synthesis were inhibited by POA. The simultaneous inhibition of synthesis of different macromolecules and serine uptake by POA is best explained by its effect on decreasing membrane potential, which is required for membrane transport. These data indicate that POA or PZA targets the membrane and interfere with the energetic and function of the membrane.

Pyrazinoic acid n-octyl ester at 100mg/ml was highly bactericidal against *M.tuberculosis*. Pyrazine thiocarboxamide at 100mg/ml was highly bactericidal against *M.tuberculosis*. N-hydroxymethyl pyrazine thiocarboxamide at 100, 50 and 25mg/ml was highly bactericidal against *M.tuberculosis* respectively.

The mode of action of Pyrazinoic acid has not been conclusively established, but it is postulated that Pyrazinamide exerts its antimicrobial activity after conversion to the active agent, POA within the bacterial cells by a bacterial enzyme. If this hypothesis is valid, it may be possible to find new drugs with a similar mode of action among PZA analogues.

This study also reveals that Pyrazinamide has a unique sterilizing activity against *M.tuberculosis*. Its unique role in tuberculosis treatment has led to the search and development of its structural analogues. One such analogue is 5-chloro-pyrazinamide (5-Cl-PZA) that has been tested under in vitro conditions against *M.tuberculosis*. The present study was designed with an aim to assess the activity of 5-Cl-PZA, alone and in combination with first line drugs, against tuberculosis.⁷



5-Chloro-Pyrazinamide

To test the tolerability of orally administered 5-Cl-PZA, uninfected mice received doses up to 300mg/Kg for 2 weeks. Four weeks after low dose aerosol infection either with *M.tuberculosis* or *M.bovis*, mice were treated 5 days/wk with 5-Cl-PZA, at doses ranging from 37.5 to 150mg/kg, either alone or in combination with Isoniazid and Rifampin. Antimicrobial activity was assessed by colony forming unit counts in lungs after 4 and 8 week of treatment.⁷

RESULTS:-

Success rates are far worse for multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB cases. There is general agreement that new anti-TB drugs are needed to shorten or otherwise simplify treatment for drug-susceptible and MDR/XDR-TB, including TB associated with HIV infection. There exists a clear and pressing need to develop new anti-TB drugs. They are needed to

- Shorten and simplify treatment of drug-susceptible TB.
- Provide shorter, safer, more effective and cheaper treatment alternatives for MDR-TB (and XDR).
- Abolish obstacles to effective treatment of TB in HIV positive individuals.

PZA is also one of the cornerstone drugs retained in the treatment of MDR-TB. WHO guidelines suggest that four effective drugs plus PZA be given for MDR-TB, irrespective of the likely effectiveness of PZA.⁸

DISCUSSION:-

PZA is a unique anti-tuberculosis drug that plays a key role in shortening the TB therapy. PZA kills non replicating persisters that other TB drugs fail to kill, and thus making it an essential drug for inclusion in any drug combinations for treating drug susceptible and drug-resistant TB such as MDR-TB. Aspartate decarboxylase (PanD) as a new target involved in PZA action. Recently, identified a new gene PanD encoding aspartate

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decarboxylase, involved in the synthesis of Panthothenate (vit B₅) which in turn required for the synthesis of CoA, a molecule that is at the center of all energy metabolism and allows carbohydrates, fats and proteins to be burned as energy sources. These findings shed new light on the mode of action of PZA and may help in the design of new drugs that shorten therapy. It has been observed that Aspirin and Ibuprofen enhances the effect of PZA during the initial phase of tuberculosis treatment in the mouse model. The Pyrazinamide-resistant *M.tuberculosis* isolates usually lose their Pyrazinamidase activity. After cloning and sequencing the gene that encodes Pyrazinamidase (PncA), it was found that 72-97% of all Pyrazinamide-resistant clinical isolates tested carry a mutation in the structured gene.⁹

CONCLUSION:-

Pyrazinamide is an important first-line tuberculosis (TB) drug used in combination with other TB drugs for the treatment of both drug-susceptible TB and multidrug-resistant tuberculosis (MDR-TB). PZA is a peculiar persister drug that acts only on dormant non-growing persisters and has poor activity against growing *M.tuberculosis*. Its high activity against persister bacteria is responsible for PZA's unique sterilizing activity, which shortens the TB treatment period from 9-12 months to 6 months. Hence we can conclude that, because of its indispensable sterilizing activity, all new TB regimens in clinical development include PZA.¹⁰

ACKNOWLEDGEMENT:-

This work was supported in part by K.V.M College of Pharmacy, Cherthala.

AUTHOR CONTRIBUTIONS:-

All authors equally contributed to this review. All authors were participated in the collection of data, preparation of manuscript and finally approved the review.

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ABBREVIATIONS:-

- TB- Tuberculosis
- MDR-TB-Multidrug resistant tuberculosis
- PZA-Pyrazinamide
- PanD-Gene encoding Aspartate decarboxylase

CONFLICT OF INTEREST REPORTED: NIL;**SOURCE OF FUNDING: NONE REPORTED**