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Mini Review

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Pyrazinamide drug resistance in *Mycobacterium tuberculosis*: a mini review

Abstract

Pyrazinamide (PZA) is a nicotinamide analog that is used as a frontline drug to treat tuberculosis (TB). It has special place in modern TB therapy, as it appears to kill a population of semi dormant tubercle bacilli persisting in the body. It is important drug because of its sterilizing activity against semi dormant tubercle bacilli. Although PZA has a very high *in vivo* activity, it's *in vitro* activity is not apparent unless an acidic environment is available, which makes PZA drug susceptibility testing (DST) more difficult by conventional methods. Being a nicotinamide analogue, PZA needs to be converted into its active bactericidal form pyrazinoic acid (POA) by the mycobacterial enzyme pyrazinamidase (PZase). PZase is encoded by *pncA*, and mutations in this gene have been demonstrated as the major mechanism of PZA resistance. PZA-resistant *Mycobacterium tuberculosis* strains are usually correlated well with defective PZase activity. The rapid detection of PZA resistance is of utmost importance for an effective treatment and also to avoid further spread of MDR strains. Analysis of the *pncA* gene provides rapid and useful information regarding PZA susceptibility in *M. tuberculosis* and may therefore contribute to early optimization of treatment. The diversity of methods currently used in clinical laboratories for the detection of PZA resistance in *M. tuberculosis* isolates causes inconsistent results of PZA DST and such inconsistent results of PZA DST have been reported by a number of laboratories by various methods, including the qualitative BACTEC test. Therefore, it is essential to elucidate the genetic basis of clinical resistance and to correlate phenotypic and molecular resistance data.

Keywords: pyrazinamide resistance, *mycobacterium tuberculosis*, qualitative bactec test, rifampicin, isoniazid

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Abbreviations: TB, tuberculosis; PZA, pyrazinamide; DST, drug susceptibility testing; POA, pyrazinoic acid; MDR, multi drug-resistant

Introduction

The addition of PZA with isoniazid and rifampicin, forms the cornerstone of modern TB therapy, shortening the TB therapy from previously 9-12months to current 6months. It kills a semi-dormant tubercle bacilli population in acidic pH environments that are not killed by any other anti- TB drugs.[1] Though PZA has a remarkable role in shortening the treatment duration, it remains a difficult paradox because of its incompletely understood mode of action as well as mechanism of resistance.[2] Basically PZA is bacteriostatic, but can become bactericidal on actively replicating tubercle bacteria.[3] It is a nicotinamide analogue, a prodrug which gets converted into the active bactericidal form pyrazinoic acid (POA) by the mycobacterial enzyme pyrazinamidase (PZase).[2] However, an exact mechanism of its action is unknown. It has been suggested that the accumulation of POA in acidic conditions (from lactic acid produced by inflammation of cells) leads to acidification of the cytoplasm and results in cell death.[4] PZase is encoded by *pncA*, and mutations in this gene have been demonstrated as the major mechanism of PZA resistance. Several mutations, including missense, insertion, deletion and nonsense mutations have been reported and located in both the putative promoter and coding regions of *pncA*.[5,6] PZA-resistant *Mycobacterium tuberculosis* strains are usually correlated well with defective PZase activity, but some PZA resistant strains have been reported to contain wild-type and to maintain PZase activity suggesting that there might be other unknown resistance mechanisms could be responsible for the resistance phenotype.[6–8]

Anti-tuberculosis drugs are known as two-edged sword. Although, these drugs are capable to destroy pathogenic *M. tuberculosis*, on the other hand, they also go for selection of drug resistant tubercle bacteria against which those drugs are ineffective. Tuberculosis (TB) global surveillance has shown that such drug resistant TB is widespread and is an alarming threat to TB control programmes in many countries.[9] Although consistent effort in monitoring and TB treatment is going on still the disease remains as a major public health issue.[10] In year 1982, the World Health Organization (WHO) estimated worldwide every year occurrence around 4-5million new highly infectious, smear-positive cases of pulmonary infection with *M. tuberculosis*. PZA was synthesized way before 1940, though its antituberculous activity was not recognized until the early 1950s.[11] WHO estimated around 8.8million new cases of TB globally in year 2003 within an expected 1% increase every year. However, appropriate therapies and rapid diagnosis become the first priorities in controlling the growing TB epidemics.[10] Even, after decades of decline, rates of TB cases are still increasing worldwide. Today TB is the most frequent cause of death from a single infectious disease in persons aged 15-49years, causing a total of 2-3million deaths annually. One- third of the human population is thought to be infected by the *M. tuberculosis*, and about 200million additional people are at risk of developing disease

in the next 20years, as emerging coinfection of TB with human immunodeficiency virus (HIV) and resistance of *M. tuberculosis* to the presently used TB regimen.[12] In both low- and high- income countries Multidrug-resistant (MDR) strains of *M. tuberculosis* have been emerging worldwide.[13,14] Since long PZA has been known to be active against pulmonary TB in humans and is used as a first-line antituberculosis drug in routine TB therapy. It's mechanism of action is least understood. Because of its outstanding sterilizing activity against semi dormant tubercle bacilli, it plays an important role in the treatment of both drug-susceptible and drug-resistant TB. The MDR-TB inclusive of PZA drug resistance definitely worsen prognoses, and the rising prevalence of such MDR-TB and HIV- MDR –TB co-infection is alarming.[4,15,16] The need for rapid methods of the diagnosis and determination of drug susceptibility testing (DST) is very important. While the procedures for DST of the most of the first-line and second-line antituberculosis drugs have been well standardized in both liquid and solid media, the main problem comes with the PZA DST, is the requirement of acidic pH for PZA activity.[17] It also becomes difficult because of the acidic pH of culture medium restricts the growth of organism. In addition, the use of large inoculum sizes results in the release of NH3 that leads to increased pH and inactivated PZA.[15] The application of various rapid phenotypic methods such as radiometric BACTEC 460, flourimetric Mycobacteria Growth Indicator Tube (MGIT) 960 (Becton Dickinson), ESP Culture System II (Trek Diagnostic Systems, West-lake, OH), and the colorimetric BacT/ALERT 3D system (bioMerieux Inc., Durham, NC), previously designated MB/BACT (Organon Teknika, Boxtels, The Netherlands) has been reported to be very useful for rapid and reliable susceptibility testing of *M. tuberculosis*isolates.[17] Presently many laboratories performing PZA liquid DST by the non-radiometric, fully automated, continuous-monitoring MGIT 960 system (Becton Dickinson) that gives reliable result with the turnaround time of 15-22days.[18,19] Number of studies revealed a good correlation between loss of PZase activity and resistance to PZA.[4,6,20,21] However, the rapid detection of PZA resistance is of utmost importance for an effective treatment and also to avoid further spread of MDR strains. Molecular assays those detect the genetic variants that mediate resistance constitute a rapid alternative to conventional DST and may even be performed directly on clinical specimens without culture. Therefore, it is essential to elucidate the genetic basis of clinical resistance and to correlate phenotypic and molecular resistance data. It is considered that the analysis of the *pncA* gene provides rapid and useful information regarding PZA susceptibility in *M. tuberculosis* and may therefore contribute to early optimization of treatment. The most PZA-resistant *M. tuberculosis* strains have mutations in the *pncA* gene has implications for developing a rapid test for detecting PZA-resistant *M. tuberculosis* strains.[21] The diversity of methods currently used in clinical laboratories for the detection of PZA resistance in *M. tuberculosis* isolates causes inconsistent results of PZA DST.[22] Inconsistent results of PZA DST have been reported by a number of laboratories by various methods, including the qualitative BACTEC test.[23]

Almost all PZA-resistant *M. tuberculosis* strains have mutations in the *pncA* gene points the necessity for developing a rapid test for detecting such PZA-resistant *M. tuberculosis* strains globally.

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Conflict of interest

The author declares no conflict of interest.

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