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# Pyrazinamide resistance in MDR and extensively drug-resistant tuberculosis.

**Lan Yao, Qing Zhang, Yidan Liu, Heping Xiao***

Tuberculosis Center for Diagnosis and Treatment, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, PR China

## Abstract

**Background: Pyrazinamide (PZA) is an important first-line antituberculosis drug in drug resistant and drug sensitive regimen. Incorporation of pyrazinamide into the first-line regimen had made it possible to reduce the treatment duration to six months. But drug resistant rate of PZA is high in MDR and XDR tuberculosis.**

**Methods: We reviewed a phase II clinical trial in our hospital to identify the clinical characteristics of PZA resistant tuberculosis.**

**Results: In our study, the mean time of anti-tuberculosis treatment before enrolled in the study was 2.6 ± 3.1 years in the PZA resistant (PZAr) group and 2.3 ± 1.4 years in the PZA sensitive (PZAs) group. The mean time of using PZA prior to the study in PZAr group and PZAs group were 1.1 ± 2.0 years and 0.5 ± 0.3 years respectively. The rate of PZA resistance was 80% (16/20) in MDR-TB and 100% (7/7) in XDR-TB.**

**Conclusions: So whether to use PZA in MDR or XDR tuberculosis regimen still needs be identified.**

**Keywords:** Tuberculosis, Pyrazinamide resistant, Multidrug resistance, MDR/XDR-TB.

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## Introduction

Pyrazinamide is an antituberculosis drug which can be dated back to 1950s. It has been estimated that pyrazinamide works by binding the ribosomal protein S1 and then inhibits the trans-translation [1]. PZA plays a central role in antituberculosis chemotherapy by making it possible to reduce the first-line treatment duration to six months in combination with isoniazid and rifampin [2]. But there is the emergence of pyrazinamide resistant strains threatening the public health, as pyrazinamide is important in both first and second-line treatment regimens [3]. WHO recommends pyrazinamide be included in the intensive phase with a fluoroquinolone, a parenteral agent, ethionamide (or prothionamide), and cycloserine, or else para-aminosalicylic acid if cycloserine cannot be used in the treatment of patients with Multi-drug-resistant tuberculosis (MDR-TB) in 2011 [4]. In recent edition of guidelines for drug-resistant tuberculosis by WHO, PZA is still recommended in the intensive phase unless there is confirmed resistance from reliable DST (WHO treatment guidelines for drug-resistant tuberculosis. 2016 update (WHO/HTM/TB/2016.04). Geneva, World Health Organization. But the recent study showed that of all the MDR strains, 69.0% (40/58) were PZA resistant [5]. To further understand the clinical characteristics of pyrazinamide resistance in MDR/XDR tuberculosis, we reviewed a phase II clinical trial in Shanghai Pulmonary Hospital.

## Materials and Methods

### *Patient recruitment*

38 MDR-TB patients were recruited in Shanghai pulmonary hospital for a phase II global multi-center trial to evaluate the safety, efficacy, and pharmacokinetics of multiple doses of delamanid (Trial 204). The 38 patients were randomly assigned to different treatment groups. Inclusion criteria were: 1) written, informed consent; 2) male or female aged between 18 and 64 years; 3) either mycobacterial culture of sputum positive for growth or sputum smear positive for acid-fast bacilli with a positive rapid test for rifampicin resistance on direct sputum, 60 days before the expected date of enrolment; 4) patients with TB caused by isolates of *M. tuberculosis* complex confirmed to be resistant to isoniazid and rifampicin; 5) findings on chest radiograph consistent with TB; 6) able to produce sputum for mycobacterial culture. The study was approved by the Shanghai Pulmonary Hospital Ethics Committee and written informed consent was obtained from all patients before enrolment.

### *Processing*

Patients enrolled underwent sputum smear examination and sputum culture in MGIT BACTEC 960 system. 27 patients were cultured positive while other 11 patients were cultured negative. Then these 27 samples had bacterial identification to exclude the non-tuberculosis mycobacteria and had Drug

Susceptibility Test (DST) of Streptomycin (Sm), Isoniazid (H), Rifampin (R), Ethambutol (E), Pyrazinamide (Z), Amikacin (Am), Capreomycin (Cm) and Ofloxacin (Ofx). The drug concentration of the medium was as follows: Sm 1.0 mg/ml, H 0.1 mg/ml, R 1.0 mg/ml, E 5.0 mg/ml, Z 100 μg/ml, Am 1.0 mg/ml, Cm 2.5 mg/ml, Ofx 2.0 mg/ml. Patients had their demography information including sex, age, medication history and drug resistance profile.

### *Statistical analysis*

The data was analysed by software SPSS19.0. Difference between the two groups was compared by using independent-samples T-test. Categorical measurements were analysed by means of the $\chi^2$ test or Fisher's exact test in the case of 2 × 2 contingency tables. We performed logistic regression analysis to explore the associations between PZA using time and the resistant to PZA outcomes. P values<0.05 were considered statistically significant.

## Results

### *Demographic characteristics*

38 patients were randomly assigned in this trial. But 11 patients had negative sputum culture, so only 27 patients with positive culture of *M. tuberculosis* were included in the research. The demographic characteristics of these 27 patients were listed in Table 1. All patients were HIV negative. 3 (11.1%) patients were female and the average age was 34.9 ± 10.6 (19-54) years. The average time of anti-tuberculosis treatment before enrolment was 2.6 ± 2.9 (1 month to 8.25 years) years. 20 samples were classified as MDR-TB and 7 samples were Extensively Drug-Resistant Tuberculosis (XDR-TB).

### *Analysis of PZAs and PZAr group*

Patients were divided into 2 groups according to DST results of PZA sensitivity (Table 2). 23 patients were resistant to PZA (PZAr group); 4 patients were sensitive to PZA (PZAs group).

There is no significant different between the two groups in age, sex, medication history and imaging performance of cavities (P>0.05). The mean time of anti-tuberculosis treatment before enrolled in the study was 2.6 ± 3.1 years in PZAr group and 2.3 ± 1.4 years in PZAs group. The mean using time of PZA prior in PZAr group and PZAs group was 1.1 ± 2.0 years and 0.5 ± 0.3 years respectively. 16 (80%) of MDR-TB isolates were resistant to PZA and all 7 (100%) XDR-TB isolates were resistant to PZA. Most patients had been used PZA for less than 6 months in both PZAr group (16/23) and PZAs group (3/4). Logistic regression analyses showed no significant associations between PZA using time and the resistant to PZA outcomes (odds ratio=0.60, 95% confidence interval=0.11-3.50).

***Table 1.*** *Demographic characteristics, drug resistance, PZA using time and average treatment time in MDR/XDR-TB patients at the baseline.*

| Case no. | Gender | Age (years) | Resistant to PZA | Drug resistance profile | PZA using time (years) | Average Treatment time (years) |
|---|---|---|---|---|---|---|
| 1 | Male | 50 | Yes | HRE | 0.58 | 0.58 |
| 2 | Male | 33 | Yes | SHREOfx | 1.5 | 2.42 |
| 3 | Male | 44 | Yes | SHREOfx | 0.17 | 3.5 |
| 4 | Male | 19 | Yes | SHREOfx | 0.42 | 1.25 |
| 5 | Male | 29 | Yes | SHREAm | 1.83 | 8.25 |
| 6 | Male | 33 | Yes | SHREAmCmOfx | 6 | 13 |
| 7 | Male | 47 | Yes | SHREAmCmOfx | 1.75 | 2.83 |
| 8 | Male | 28 | Yes | SHREOfx | 8.25 | 8.25 |
| 9 | Female | 21 | Yes | SHROfx | 0.83 | 2.42 |
| 10 | Male | 19 | Yes | SHREOfx | 0.42 | 0.75 |
| 11 | Male | 45 | Yes | SHREOfx | 0.08 | 0.08 |
| 12 | Male | 42 | Yes | SHREAmCmOfx | 0.17 | 2.17 |
| 13 | Male | 42 | Yes | SHREAmCmOfx | 0.25 | 3.33 |
| 14 | Male | 47 | Yes | SHREOfx | 0.5 | 2 |
| 15 | Male | 22 | Yes | HRE | 0.17 | 0.92 |
| 16 | Female | 24 | Yes | SHREAmOfx | 0.58 | 0.58 |
| 17 | Male | 52 | Yes | SHREAmCmOfx | 1.08 | 2.08 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 18 | Female | 22 | Yes | SHREOfx | 0.25 | 0.92 |
| 19 | Male | 27 | Yes | SHREAmOfx | 0.33 | 1.58 |
| 20 | Male | 35 | Yes | HR | 0.25 | 0.25 |
| 21 | Male | 27 | Yes | SHRE | 0.25 | 0.58 |
| 22 | Male | 33 | Yes | SHRE | 0.17 | 0.67 |
| 23 | Male | 54 | Yes | HROfx | 0.25 | 2.08 |
| 24 | Male | 32 | No | SHR | 0.33 | 4 |
| 25 | Male | 33 | No | HRE | 0.83 | 1.5 |
| 26 | Male | 37 | No | HR | 0.58 | 2.83 |
| 27 | Male | 44 | No | SHR | 0.25 | 0.75 |

Notes: S: Streptomycin; H: Isoniazid; R: Rifampin; E: Ethambutol; Am: Amikacin; Cm: Capreomycin; Ofx: Ofloxacin.

***Table 2.*** *Analysis of the PZAs and PZAr group.*

| | PZAs group | PZAr group | P |
|---|---|---|---|
| Patient number | 4 | 23 | |
| Age, y, mean | 36.5 ± 5.4 | 34.6 ± 11.3 | 0.065 |
| Sex | | | 1 |
| Male | 4 | 20 | |
| Female | 0 | 3 | |
| Average treatment time (years) | 2.3 ± 1.4 | 2.6 ± 3.1 | 0.825 |
| PZA using time (years) | 0.5 ± 0.3 | 1.1 ± 2.0 | 0.535 |
| ≤ 6 months | 3 (75%) | 16 (69.6%) | |
| >6 M ≤ 1 y | 1 (25%) | 1 (4.3%) | |
| >1 y ≤ 5 y | 0 (0%) | 4 (17.4%) | |
| >5 y | 0 (0%) | 2 (8.7%) | |
| With cavity | 4 (100%) | 22 (95.7%) | 1 |
| Drug resistant to | | | |
| Ethambutol | 1 (25%) | 18 (78.3%) | 0.065 |
| Streptomycin | 2 (50%) | 19 (82.6%) | 0.204 |
| Amikacin | 0 (0%) | 8 (34.8%) | 0.285 |
| Capreomycin | 0 (0%) | 5 (21.7%) | 0.561 |
| Ofloxacin | 1 (25%) | 17 (73.9%) | 0.093 |
| MDR | 4 (100%) | 16 (69.6%) | 0.545 |
| XDR | 0 (0%) | 7 (30.4%) | |

## Discussion

The most popular accepted mechanism of pyrazinamide is that it converses into pyrazinoic acid by the bacterial pyrazinamidase enzyme then be expelled from the bacteria by an efflux pump [6]. The pyrazinoic acid is protonated in the extracellular environment and then re-enters the mycobacterium, releasing the proton and causing a lethal disruption of the membrane [3]. So that pyrazinamide can kill semi dormant tuberculosis bacteria existing in the macrophages with acidic environments while other drugs cannot do so well there [6].

Although WHO recommends pyrazinamide to be an essential drug in the intensive phase, the recommendation is conditional and the quality of evidence is very low [4]. WHO also recommends the radiometric (Bactec 460TB) or non-radiometric (Bactec MGIT 960) liquid medium to test PZA susceptibility, with a MIC of 100 mg/l considered susceptible and otherwise resistant. Whitfield et al. [7] summarized several systematic reviews and meta-analysis to find that the PZA resistant rate increases with the resistance to other drugs. In one review, Chang et al. [8] identified 128 articles with eligible pyrazinamide susceptibility testing in *Mycobacterium tuberculosis* and the author found that the median prevalence (range) of pyrazinamide resistance was much higher in MDR tuberculosis isolates (51% (31% to 89%)) than in non-MDR isolates (5% (0% to 9%)) [8]. Astonishingly, more than 90% of the XDR-TB strains tested showed phenotypic resistance to pyrazinamide in Europe by a study of patients with MDR, pre-MDR and XDR-TB in 16 European countries [9]. A recent study indicated that non-MDR isolates had PZA resistant rate of only 11.7% (12/103) while it is 69.0% (40/58) in all the MDR isolates by gene sequencing detection [5]. This is the same in our study; the PZA resistant rate was 80% (16/20) in MDR-TB and 100% (7/7) in XDR-TB. The drug resistant prevalence in PZAr MDR patients in this study were 82.6% (19/23) for streptomycin, 78.3% (18/23) for ethambutol, 34.8% (8/23) for amikacin, 21.7% (5/23) for capreomycin and 73.9% (17/23) for ofloxacin.

With such a high resistant rate to PZA, is this drug still effective in treating MDR-TB? The review by Ahuja et al. [10] used random effects multivariable logistic meta-regression to estimate adjusted odds of treatment success and found that treatment success, compared to failure/relapse or death, was significantly associated with the use of PZA (aOR 1.3, 95% CI 1.1-1.6). But when compared to failure or relapse, the aOR was

1.2, (95% CI 0.9-1.7), and aOR was 1.1 (95% CI 0.9-1.4) compared to failure, relapse, death or default. It showed that the association between use of PZA and treatment success was not strong in this review. But the author did not compare the treatment success between PZA resistant subjects and PZA sensitive subjects. In another review including MDR-TB and XDR-TB, PZA susceptible patients using PZA was significantly associated with treatment success (aOR 1.6, 95% CI 1.1-2.4) *vs.* failure or relapse, and aOR 1.4, 95% CI 1.1-1.8 *vs.* failure, relapse or death) [11]. In the study of Chang et al. [12] assembled a cohort of 194 patients with MDR pulmonary TB given fluoroquinolone-containing regimens. Authors suggest that pyrazinamide is very important in fluoroquinolone-based tuberculosis therapy because when combined PZA in PZA sensitive patients increases the early sputum culture conversion rate and treatment success rate of 38% for both by a best estimate. But there was also a different conclusion. Budzik et al. [13] found that treatment failure and mortality rates were non-significantly different between MDR-TB cases with PZA-resistant and PZA-susceptible MDR-TB controls (18% *vs.* 29%). There was no statistically significant difference between treatment failure and mortality rates in patients infected with PZA monoresistance compared to pansusceptible controls (4% *vs.* 8%, p=0.51), or those with PZA and MDR resistance compared to MDR controls (18% *vs.* 29%, p=0.40). In our study, the patients were assigned to different groups with different dose of delamalid, so the relationship between PZA susceptibility and treatment result was difficult to decide.

Are there any characteristics associated with PZA resistance in patients with MDR-TB? Budzik et al. [13] discovered that there were no significant differences in patient characteristics between PZA and MDR resistance groups and MDR groups. In our study, we calculated the previous PZA using time and the previous average anti-tuberculosis treatment time of the two groups. The mean time of anti-tuberculosis treatment was 2.6 ± 3.1 years in the PZAr group and 2.3 ± 1.4 years in the PZAs group before enrolment. The mean time of using PZA in the PZAr group and PZAs group were 1.1 ± 2.0 years and 0.5 ± 0.3 years respectively before enrolment. 16 (80%) of MDR-TB samples were resistant to PZA and all 7 (100%) of XDR-TB samples were resistant to PZA. But the case number was too small to give an appropriate statistical analysis to calculate the relationship between the using time and PZA resistance. Most patients (69.6%) used PZA for less than 6 months in the PZAr group before enrolment. Does this mean the PZA resistant tuberculosis inclined to develop into MDR-TB? To solve this conjecture we need carefully planned cohort study with large sample sizes and long follow-up duration.

In 2014, 480 000 cases of multidrug-resistant TB estimated to have occurred, and an estimated 190 000 people died of MDR-TB. Patients with drug-resistant TB currently require a minimum of 18 to 24 months of treatment. During the long treatment period, patients have to take more than 14,000 pills of different kind and injections daily for at least 6 months in the intensive phase [14]. The long and more aggressive MDR-TB treatment brings the pain and side effects which may lower the treatment compliance [14]. Now we have two brand-new antituberculosis drugs on the market, bedaquiline and delamanid, which have shown effectiveness in preliminary studies of shortened regimens treating MDR tuberculosis [15]. Incorporation of pyrazinamide and other newly invented drugs into a new regimen had been tested with satisfactory result. Some other new drugs are in the process of phase II or phase III clinical trials. For example, Pretomanid, a nitroimidazole new drug, when combination with moxifloxacin and pyrazinamide, was safe, well tolerated, and showed superior bactericidal activity in MDR tuberculosis during 8 weeks of treatment [16]. This new regimen may enter phase III trial to shorten and simplify treatment of drug-susceptible and MDR tuberculosis. There were also other combinations of new drugs with PZA which have showed efficacy [17]. So eventually we still depend on the trials to give us the result of the benefit to add PZA in a MDR regimen.

In conclusion, this is a descriptive essay with small sample size. We find that PZA resistant rate increases in prevalence as risk of resistance to other drugs increases. In our study, the rate of PZA resistance was 80% (16/20) in MDR-TB and 100% (7/7) in XDR-TB. The mean time of using PZA prior to the study in PZAr group and PZAs group were 1.1 ± 2.0 years and 0.5 ± 0.3 years respectively. It is difficult to decide if the PZA resistance can induce more drug resistance or result in bad treatment outcome. And is it useful for MDR-TB patients to take PZA when DST showed resistant. So case control study of large sample sizes should be conducted to answer these questions.

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## *Correspondence to

Heping Xiao

Tuberculosis Center for Diagnosis and Treatment

Shanghai Pulmonary Hospital

Tongji University School of Medicine

PR China