

Investigation of Cross-Resistance between Rifampin and Rifabutin in Multi-Drug Resistant *Mycobacterium tuberculosis*

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Abstract

Objective: In order to indicate the cross-resistance between rifampin (RFP) and rifabutin (RFB) in multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB) clinical strains and to provide the laboratory data for using rifabutin in the treatment of MDR-TB.

Methods: The minimal inhibitory concentrations (MICs) of RFB and RFP in 7H10 Middlebrook medium against 99 MDR-TB clinical strains were determined by microplate assays.

Results: Among these 99 isolates, 85 were resistant to rifabutin at concentrations $>0.5 \mu\text{g/ml}$. The cross-resistance ratio between rifampin and rifabutin was 85.86%. The MICs of RFB were 8-32 times lower than those of RFP ($\chi^2=125.905$, $p<0.001$). The cross-resistance ratio increased with the resistance level of RFP. The cross-resistance strains in the lower (the MICs of RFB $2 \sim 4 \mu\text{g/ml}$) and the medium groups (the MICs of RFB $8 \sim 16 \mu\text{g/ml}$) were 0/9 and 5/9 respectively, while in the high rifampin-resistant group (the MICs of RFB $\geq 32 \mu\text{g/ml}$) almost all of the strains but one were cross-resistant (98.8%, 80/81).

Conclusion: RFB has the activities against MDR-TB clinical strains *in vitro*. The cross-resistance ratio between rifampin and rifabutin increased with the resistance level of RFP. RFB is one of alternatives for the treatment of MDR-TB.

Keywords: Rifabutin; Rifampin; *Mycobacterium tuberculosis*; Multi-drug resistance; Minimal inhibitory concentrations

Introduction

Tuberculosis (TB) currently remains a major health issue worldwide [1]. In 2014, 3.5% of new and 20.5% of previously treated TB cases was estimated to have Multidrug-resistant TB (MDR-TB), defined as resistance to at least isoniazid and rifampin, and 9.0% of patients with MDR-TB had extensively drug-resistant TB (XDR-TB) globally. XDR tuberculosis is defined as tuberculosis with resistance to at least isoniazid, rifampin, a fluoroquinolone, and 1 of 3 injectable second-line drugs (amikacin, kanamycin, or capreomycin). While the alarming increase of MDR-TB and XDR-TB may hinder current advances in TB control, new anti-TB drugs or more effective therapeutic measures are urgently needed. Rifabutin (RFB) is the emergence of derivatives of rifampin (RFP) in recent years. RFB has strong antibacterial activity against *Mycobacterium tuberculosis* (MTB) and *Mycobacterium nontuberculosis* (NTM), especially *M. avium* complex (MAC) [2]. Compared with rifampin, RFB causes fewer interactions with antiretroviral drugs, which is of relevance for HIV/TB coinfection [3,4]. With the advent of RFB, the effect of it on resistant *Mycobacterium tuberculosis* strains attracted the high attention of scholars. Related researches have been carried out. But study about the cross-resistance ratio between the two rifamycin derivatives is rare [5-9].

Our aim was to indicate the cross-resistance between rifampin (RFP) and rifabutin (RFB) in multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB) clinical strains, and to provide the laboratory data for using RFB in the treatment of MDR-TB.

Materials and Methods

Test isolates

A total of ninety-nine clinical isolates of multidrug-resistant *M. tuberculosis* strains included in this study were selected from Shanghai Pulmonary Hospital, Tongji University School of Medicine. The isolates were obtained from sputum after culture with BACTEC MGIT 960 method and then were identified by biochemical tests. A strain of H37Rv (*M. tuberculosis* ATCC 27294), gift of the National Tuberculosis Reference Laboratory (Beijing, China) was used as control.

Antimicrobial agents

The antimicrobial agents RFB, RFP were purchased from Sigma Chemical Company (St Louis, MO) which were prepared in the manufacturer-recommended solvent to a stock solution concentration of $10,000 \mu\text{g/ml}$ according to manufacturers' instructions and stored at -80°C until use.

Liquid culture medium

Liquid culture medium was Middlebrook 7H9 liquid culture containing 10% OADC enrichment ([Becton Dickinson Co., USA], the mixture of antimicrobial agents and growth indicator). Middlebrook 7H9 liquid culture was prepared according to the literature [10,11].

Inoculum preparation

M. tuberculosis suspension in log-phase growth was adjusted to 1 mg/ml . When aliquot of $100 \mu\text{l}$ *M. tuberculosis* suspension was added to 10 ml liquid culture medium, the final concentration was $10\text{-}3 \text{ mg/ml}$.

Antimycobacterial susceptibility testing

Minimum inhibitory concentration (MIC) of RFB and RFP as single agent was examined using the microwell plate method. Before use, aliquot of $100 \mu\text{l}$ liquid culture medium contained RFB or RFP dilutions was prepared and added to the sterile 96-well polystyrene U-bottom microdilution tray. The concentration range of RFB or RFP was from $0.5 \mu\text{g/ml}$ to $64 \mu\text{g/ml}$. $100 \mu\text{l}$ suspension of *M. tuberculosis* was inoculated. The final concentration range of RFB or RFP was from $0.25 \mu\text{g/ml}$ to $32 \mu\text{g/ml}$. Two drug-free controls were inoculated with

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the same suspensions diluted 1:1, 1:10 respectively; the negative control was sat at the same time. After inoculation, the culture media was incubated at 37°C. The results were observed daily after seven days to fourteen days. The appearance of visible white bacterial precipitation in the bottom of the well indicates positive. The MIC of RFB or RFP is the lowest concentration inhibiting the visible growth of the bacteria. The critical test concentration for RFB-resistant strains was 0.5 µg/ml and for RFP was 1 µg/ml according to previous reports [12,13].

Statistical analysis

Statistical analyses were conducted using SPSS statistical software (SPSS Statistics 17.0; SPSS, Inc., Chicago, IL, USA). Comparisons of positive rates between different groups were analyzed using Pearson's χ^2 test.

Results

The RFB MIC distribution for the tested 99 MDR-TB clinical isolates was from <0.25 to 16 µg/ml (median 2 µg/ml) and the RFP MIC distribution was from 2 to >32 µg/ml (median 12 µg/ml), respectively. The difference showed statistically significant ($\chi^2=125.905$, $p<0.001$). The MIC of RFB was 1/8-1/32 of RFP, which are detailed in Table 1. The distribution curve of MIC of RFB and RFP was shown in Figure 1. Of the 99 clinical isolates tested, 14 of which were susceptible to RFB, 85 were resistant to RFB. The cross-resistant ratio between RFP and RFB was 85.86% and increased with the resistant level of RFP. For strains with a RFP MIC of 2 ~ 4 µg/ml, all were susceptible to RFB. For strains with a RFP MIC of 8 ~ 16 µg/ml, 44.44% were susceptible to RFB. For strains with a RFP MIC of ≥ 32 µg/ml, only 1(1.82%) was susceptible to RFB.

Discussion

There are some studies on cross-resistance to RFB and RFP. They showed different results [5-9]. It ranged from 54% to 88% [5-7]. But for the study of MDR-TB, we only found two articles with the results of 87% and 73%, respectively [8,9]. The cross- resistance ratio in this study was 85.86%. The difference may be due to the different research methods. We used liquid culture medium in this study to avoid drug explanation [14]. In the study of Senol and Saribas, test was conducted using the proportion method on L-J medium to RFB mono-resistant isolates and some MDR-TB.

We also found that the cross- resistance ratio between RFB and RFP rose with the degree increase of RFB resistance, which is consistent with other research [15]. About half of the moderate RFP-resistant MTB and almost all of the low RFP-resistant MTB were still susceptible to RFB. Previous studies have shown that certain mutations in the *rpoB* RRDR are more likely to confer higher levels of RFP resistance. At the same time, certain *rpoB* mutations are more likely to confer lower levels of RFP resistance. Jamieson et al. reported that high MICs for RFP and RFB are associated with specific mutations at codons 531 and 526, while mutations at other positions are generally associated with low or moderate MICs. But it's a pity that we have not done related research for many reasons [16].

An important finding of this research is that the RFB MIC of the 99 MDR-TB isolates tested was much lower than RFP MIC (1/8-1/32). This is likely because RFB have higher affinity with the β -subunit of the bacterial RNA polymerase encoded by the *rpoB* gene [17]. We speculated that in clinical practice MDR-TB patients may be treatable with RFB. Unfortunately, there are very limited published data supporting the successful treatment of MDR-TB with RFB [18-21].

Subgroup	Isolates	RFB-MIC (µg/ml)						
		<0.25	0.5	1	2	4	8	16
RFP-MIC2 ug/ml	4	4	0	0	0	0	0	0
RFP-MIC4 ug/ml	5	4	1	0	0	0	0	0
RFP-MIC8 ug/ml	3	2	0	1	0	0	0	0
RFP-MIC16 ug/ml	6	0	2	4	0	0	0	0
RFP-MIC32 ug/ml	26	0	0	18	8	0	0	0
RFP-MIC>32 ug/ml	55	1	0	13	32	5	2	2

Table 1: RFP MIC and RFB MIC for MDR-TB.

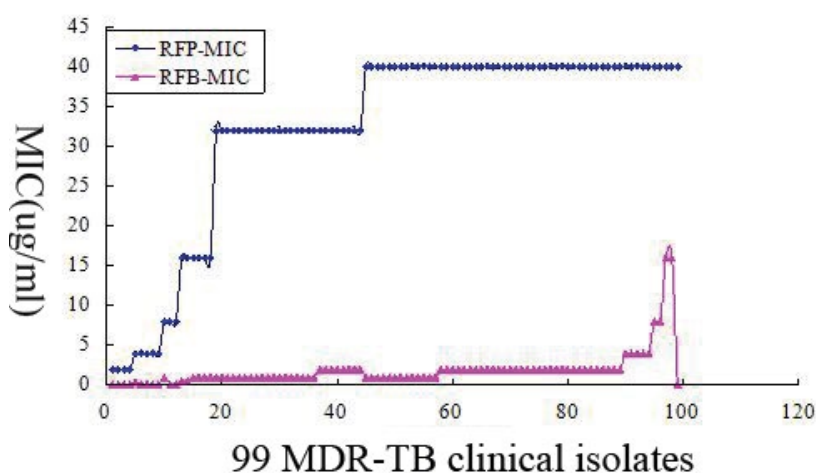


Figure 1: MIC distribution for rifabutin and rifampin in 99 MDR-TB clinical isolates.

However, the synergistic effect is crucial for assessing the effectiveness of the anti-tuberculosis chemotherapy. In our another study of *in vitro* synergism of RFB with Moxifloxacin(Mfx) and Pasiniazid(PA) on ten MDR-TB and ten XDR-TB by a three-dimensional checkerboard in Middlebrook 7H9 broth microdilutions, RFB did not show strong synergistic effect with Mfx and PA ($P=0.003$) [22]. What exactly is now required to be investigated is the synergistic effect of RFB with other anti-tuberculosis drugs.

We conclude that about half of the moderate RFP-resistant MTB and almost all of the low RFP-resistant MTB were still susceptible to RFB. When MTB was lowly resistant to RFP (MIC 2-4 µg/ml), RFB was an alternative to RFP for DR-TB patients. When MTB was moderately resistant to RFP (MIC 8-16 µg/ml), RFB was recommended only in the case of deficiency of sensitive drug to compose DR-TB chemotherapy scheme. When MTB was highly resistant to RFP (MIC ≥ 32 µg/ml), RFB was not recommended clinically.

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