Research Article Open Acces

Keywords: Multidrug-resistant M. tuberculosis; Extensively drug-resistant M. tuberculosis; In vitro synergy; Fractional inhibitory concentration index; Broth microdilution checkerboard

Introduction

Drug-resistant TB (DR-TB) is a major threat worldwide today. e main source of drug-resistant pulmonary tuberculosis is retreatment patients [1]. According to the survey in 72 countries and territories around the world, the rate of DR-TB in retreatment pulmonary tuberculosis patients with sputum smear positive was 0.0%-85.9% and the rate of multidrug-resistant tuberculosis (MDR-TB) was 0.0%-62.5%. In China, the rate of DR-TB in retreatment pulmonary tuberculosis patients with sputum smear positive was 55.17% and the rate of MDR-TB was 25.64% [2]. While DR-TB is a formidable obstacle to e ective TB care and prevention globally, the more e ective therapeutic regimen for retreatment pulmonary tuberculosis is urgently needed. However, the synergistic e ect is crucial for assessing the e ectiveness of the anti-tuberculosis chemotherapy [3-5]. Moxi oxacin(Mfx), Pasiniazid(Pa), Rifabutin(R) and Rifapentine(R) were core drugs of the national key project for infectious diseases (the retreatment research of tuberculosis). ese drugs have been carried out in the clinical application and have appeared as promising new anti-TB therapies in patients with resistance to classical drugs. But there has not been report on the synergism of these drugs. To address this need, we conducted this study of in vitro synergism of these drugs on twenty DR-MTB clinical isolates including ten MDR-TB and ten XDR-TB by a three-dimensional checkerboard in Middlebrook 7H9 broth microdilutions. We calculated the fractional inhibitory concentration index (FICI) of anti-tuberculosis drug combinations (MfxPa, MfxPaR and MfxPaR) for these isolates and judge the synergism of these drugs.

Materials and Methods

Test isolates

A total of twenty clinical isolates of M. tuberculosis including ten multidrug-resistant strains (isolate Nos.1-10) and ten extensively drug-resistant strains (isolate Nos.11-20) were included in this study from

*Corresponding author: Heping Xiao, Department of Tuberculosis, Shanghai Pulmonary Hospital, Tongji University School of Medicine, 507 Zhengmin Rd., Shanghai 200433, People's Republic of China, Tel: 86-21-65115006; Fax: 86-21-65111298; E-mail: xiaoheping_sars@163.com

Received December 17, 2014; Accepted January 06, 2015; Published January 15, 2015

Citation: Yan L, Zhang L, Yang H, Xiao H (2015) In Vitro Synergism Testing Of Three Antimicrobial Agents against Multidrug-Resistant and Extensively Drug-Resistant Mycobacterium Tuberculosis by Checkerboard Method. J Mol Pharm Org Process Res 3: 123. doi: 10.4172/2329-9053.1000123

Antimicrobial agents

e antimicrobial agents Pa, R , R were purchased from Sigma Chemical Company (St Louis, MO). Mfx was purchased from Bayer Pharmaceutical Co. Ltd. Initial stock solutions of these antimicrobial agents were prepared according to manufacturers' instructions and stored at-70°C until use [6].

Liquid culture medium

Liquid culture medium was Middlebrook 7H9 liquid culture containing 10% OADC enrichment ([Becton Dickinson Co., U.S.A.], the mixture of antimicrobial agents and growth indicator). Middlebrook 7H9 liquid culture was prepared according to the literature [7,8].

Inoculum preparation

M. tuberculosis suspensions in log-phase growth were adjusted to an optical density of 1.0 McFarland standard in sterile saline, corresponding to a cell density of approximately 10^7 colony forming units (cfu/ml). e cell suspensions were then subjected to ten-fold serial dilutions to give a nal concentration of 10^5 cfu/ml at the time of inoculation.

Antimycobacterial susceptibility testing

Minimum inhibitory concentration (MIC) of R and R as single agent was examined using the microwell plate method. Before use, aliquot of 20ul liquid culture medium contained R or R dilutions was prepared and added to the sterile 96-well polystyrene U-bottom microdilution tray. e concentration range of R or R was from 0.15 µg/ml to 320 µg/ml. When 200 µl suspension of M. tuberculosis was inoculated, the nal concentration range was from 0.015 µg/ml to 32 µg/ml. ree drug-free controls were inoculated with the same suspensions diluted 1:1, 1:10 and 1:100 respectively. e MIC of R or R is the lowest concentration causing visible white bacterial precipitation in the bottom of the well less than that of the 1:10 drug-

as being indi erent, with FICI ranging from 0.5 to 1.0. Only two isolates revealed synergy with FICI <0.5, as shown in (Table 2).

ree-agent checkerboard assay of PaMfxR and PaMfxR combination

As shown in Table 2, the combination of PaMfxR and PaMfxR was interpreted as being synergy to most of the tested MDR-TB and XDR-TB isolates, especially PaMfxR combination. PaMfxR combination showed synergism against two MDR 0 10 42.5197 692.9248 Tm [(r)8 (etidrug-Resistant and)24.8 ( )]TJ 0.164 Tw -15.4irg

is necessary to do more research on this method to investigate the application value.

Funding: is study was funded by the National Key Project for Infectious Disease (No. 2013ZX10003009)

References

1. Rusen I D (2009) Tuberculosis retreatment: a topic whose time has come. Int J Tuberc Lung Dis 13: 1192.
2. (2010) Ministry of Health of the People's Republic of China. Baseline survey report of national TB drug resistance (2007-2008). First edition. People's Medical Publishing House 49.
3. Bhusal Y, Shiohira CM, Yamane N (2005) Determination of in vitro synergy when three antimicrobial agents are combined against Mycobacterium tuberculosis. Int J Antimicrob Agents 26:292-297.
4. Rodriguez Díaz JC, Ruiz M, López M, Royo G (2003) Synergic activity of ÀXURURTXLQROROQHV DQG OLQH]ROLG DJDLQVW Antimicrob Agents 21:354–356.
5. Rey-Jurado E, Tudo G, Martínez JA, González-Martín J (2012) Synergistic effect of two combinations of antituberculous drugs against Mycobacterium tuberculosis. Tuberculosis (Edinb) 93:260-263.
6. Sanders CA, Nieda RR, Desmond EP (2004) Validation of the use of Middlebrook 7H10 ager, BACTEC 960, and BACTEC 460 12B media for testing WKH VXVFHSWLELOLW\ RI 0\FREDFWHULXP WXEHUFXORVLV LQ 0\FREDFWHULXP WXEHUFXORVLV [J]. J Clin Microbiol 43:5225-5228.
7. Rey-Jurado E, Tudó G, de la Bellacasa J P, Espasa M, González-Martín J (2013) In vitro effect of three-drug combinations of antituberculous agents against multidrug-resistant Mycobacterium tuberculosis isolates.Int J Antimicrob Agents 41: 278-280.
8. Dorman SE, Johnson JL, Goldberg S, Muzanye G, Padayatchi N, et al. (2009) 6XEVWLWXWLRQ RI PR[LÀR[DFLQ IRU LVRQLD]LG GX pulmonary tuberculosis. Am J Respir Crit Care Med 180: 273–280.
9. Moody JA (1992) Synergism testing:broth microdilution checkerboard and broth macrodilution methods. Washington, DC:American Society for Microbiology 5: 22.
10. %DB6WDKOPDQQ 5 .XELQ 5 &KRXGKUL 6 2ZHQV RI RUDO DQG LQWUDYHQRXV PR[LÀR[DFLQ FXPXOD potmarketing studies. Clini Thera 26: 940-950.
11. 3HQ'J :DQJ ' /L + &OLQLFDO REVHUYDWLRQ RI Ethambutol+Streptomycin for pulmonary tuberculosis complicated with chronic hepatitis B.Chinese Journal of clinicians 4: 920-923.
12. Heping Xiao, Shenjie Tang (2009) Clinical application of second-line anti TB drugs. Chinese Journal of Antituberculosis 31:612-616.
13. Wehrli W, Nuesch J, Knusel F (1968) Action of rifamycins on RNA Polymerase[J]. Biochim Biophys Acta 157:215-217.
14. Jarvis B, Lamb HM (1998) Rifapentine. Drugs 56: 607.
15. Dickinson JM, Mitchison DA (1987) In vitro properties of rifapentine (MDL473) relevant to its use in intermittent chemotherapy of tuberculosis[J]. Tubercle 68: 113-118.
16. Keung A, Eller MG, McKenzie KA, Weir SJ (1999) Single and multiple dose pharmacokinetics of rifapentine in man part[J]. Int J Tuberc Lung Dis 3: 437-444.