International Journal of Antimicrobial Agents 43 (2014) 148–153

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

Activity of linezolid-containing regimens against multidrug-resistant tuberculosis in mice

Weijie Zhao[a,*,1], Zhenyong Guo[b,1], Meiqin Zheng[b], Jinfu Zhang[b], Bin Wang[a], Peng Li[b], Lei Fu[a], Shuo Liu[b]

[a] Department of Pharmacology, Beijing Key Laboratory of Drug Resistance Tuberculosis Research, Beijing Tuberculosis and Thoracic Tumor Research Institute, Beijing, China
[b] Pharmacy of Beijing Chest Hospital affiliated to The Capital Medical University, Beijing, China

ARTICLE INFO

Article history:
Received 4 July 2013
Accepted 14 October 2013

Keywords:
Linezolid
Combination regimen
Multidrug-resistant tuberculosis
Extensively drug-resistant tuberculosis
Activity
Cytokine

ABSTRACT

The objective of the present study was to compare the activities of regimens containing linezolid (LZD) with those not containing LZD against *Mycobacterium tuberculosis* infection in mice. The three regimens excluding LZD selected in this study are often used in practice against multidrug-resistant tuberculosis (MDR-TB). When LZD was added to these MDR-TB regimens, the combinations were significantly more active after 2 months of therapy with regard to lung CFU reductions. The activity of LZD-containing regimens was greater than the World Health Organization's standard first-line regimen of rifampicin + isoniazid + pyrazinamide. In particular, when LZD was included in the combination levofloxacin + amikacin + *para*-aminosalicylic acid + pyrazinamide + clofazimine, culture negativity of the lungs was reached after 2 months of treatment in every case. In addition, the serum levels of interleukin-10 and interferon-γ of mice were determined and were found not to be surrogate markers of bacterial clearance.

1. Introduction

Pulmonary tuberculosis (TB) is the world's leading cause of death from a single infectious agent. Although the World Health Organization (WHO) has been working to reduce the number of TB cases, the numbers of multidrug-resistant TB (MDR-TB) cases, especially extensively drug-resistant TB (XDR-TB) cases, are rising, increasingly compromising TB control. In 2012, the WHO reported more than 400,000 new cases of MDR-TB [1]. MDR-TB [TB resistant to isoniazid (INH) and rifampicin (RIF)] and XDR-TB (resistant to INH, RIF, any fluoroquinolone and at least one of three injectable second-line drugs) are major causes of morbidity and mortality worldwide. Currently there is no single standard MDR-TB regimen as is the case with the short-course treatment regimen(s) for drug-susceptible TB, mainly because of varying drug susceptibility patterns in patients with MDR-TB. The WHO has not recommended a regimen other than a basic principle that one injectable drug and one fluoroquinolone should be included in the combinations composed of four to five agents in general. Other second-line anti-TB agents should be considered if the *Mycobacterial tuberculosis* isolate is found to be resistant to fluoroquinolones [2]. Based on these rules, regimens against MDR-TB were selected always according to the experience of physicians and the status of patients. Whatever regimens are selected, the lower activity [3] and safety of these regimens are still not optimal because the treatment duration is too long (≥20 months) [2,4]. This calls for new powerful combinations to shorten the duration of current TB treatment and to treat MDR-TB and XDR-TB [5].

Recent studies have found that linezolid (LZD) has good activity against MDR-TB, with a minimum inhibitory concentration (MIC) range of 0.06–1 mg/L and a MIC_{90} value (MIC for 90% of the organisms) of 0.5 mg/L [6]. With excellent results for medium- and long-term treatment of patients [7,8], LZD also displayed favourable effects against MDR-TB, with a treatment success rate of 67.99% (95% confidence interval 58.00–78.99%), although the data were limited [9], with similar results in XDR-TB [7]. However, because such patients were treated concomitantly with other anti-TB drugs, often including aminoglycosides and/or fluoroquinolones, it has been difficult to assess the contribution of LZD to their treatment outcomes. In this study, the efficacies of three regimens often used in clinical practice as well as the activities of these regimens when combined with LZD against MDR-TB in a murine model infected with a high dose of *M. tuberculosis* were evaluated. The regimens

* Corresponding author. Present address: No. 97 Beimachang, Tongzhou District, Beijing 101149, China. Tel.: +86 10 8950 9358; fax: +86 10 6954 6480.
E-mail address: zhaoweijie415@163.com (W. Zhao).
[1] These two authors contributed equally to this manuscript.

0924-8579/$ – see front matter.
http://dx.doi.org/10.1016/j.ijantimicag.2013.10.010

selected in this study include: (i) levofloxacin (LFX) + amikacin (AMK) + *para*-aminosalicylic acid (PAS) + ethambutol (EMB); (ii) AMK + PAS + pyrazinamide (PZA) + prothionamide (PTO); and (iii) LFX + AMK + PAS + PZA + clofazimine (CFZ).

Interferon-γ (INFγ) and interleukin-10 (IL-10) play an important role in the development of TB, body defences, inflammation and tissue repair. Secreted by Th1 cells, INFγ activates macrophages to kill bacteria. Peripheral blood levels of INFγ in mice reached a peak 20 days after being infected with *M. tuberculosis* and declined as treatment progressed [9,10]. INFγ levels correlated with the number of bacteria in the lungs [11]. IL-10 secreted by macrophages and T-lymphocytes upon infection can suppress the immune response and promote the development of the disease. It was reported that saliva levels of IL-10 in TB patients declined after 30 days of treatment while the Th1 response increased [12]. Therefore, levels of INFγ and IL-10 in peripheral blood of mice were determined to evaluate the activities of regimens against *M. tuberculosis*.

2. Materials and methods

2.1. Antimicrobial agents

The compounds used were purchased from various manufacturers. LZD was from Pfizer Pharmaceuticals (Caguas, Puerto Rico), LFX was from Shuanghe Pharmaceutical Co., Ltd. (Beijing, China), CFZ was from Nanjing Liye Pharmaceutical Co., Ltd. (Nanjing, Jiangsu, China), PAS and PZA were from Shanghai Xinyi Pharmaceutical Co., Ltd. (Shanghai, China), RIF, INH, AMK and EMB were from Sigma (St Louis, MO) and PTO was from Kangboshi Pharmaceutical Co., Ltd. (Anshan, Liaoning, China). AMK was diluted in normal saline. The remaining drugs were suspended at the desired concentrations in distilled water containing 0.05% agar. Drug suspensions were prepared weekly and were stored at 4 °C.

2.2. Determination of minimum inhibitory concentrations

Organisms were grown at 37 °C with 5% ambient CO_2 for 14 days in Middlebrook 7H9 broth (Becton Dickinson, Sparks, MD) supplemented with 0.2% (v/v) glycerol (Sigma), 10% (v/v) oleic acid–albumin–dextrose–catalase (OADC) (Becton Dickinson) and 0.05% (v/v) Tween 80 (Sigma). Bacteria were washed and suspended in phosphate-buffered saline and were passed through an 8-μm pore size filter to eliminate clumps. The filtrates were aliquoted, stored at −80 °C and were used within 30 days.

MICs of all of the antimicrobial agents except PZA against *M. tuberculosis* H37Rv (ATCC 27294) were determined by the microplate Alamar blue assay [13]. Briefly, serial two-fold concentrations of drugs were used to determine the MICs of these agents against H37Rv. Final drug concentration ranges were as follow: RIF, 0.0125–0.4 mg/L; INH, 0.0125–0.4 mg/L; LFX, 0.125–2 mg/L; LZD, 0.0625–2 mg/L; PAS, 0.0625–2 mg/L; CFZ, 0.06–0.96 mg/L; AMK, 0.125–4 mg/L; PTO, 0.25–8 mg/L; and EMB, 0.5–16 mg/L. The MICs for H37Rv are shown in Table 1.

2.3. Aerosol infection

Male 6-week-old BALB/c mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China) and were infected with *M. tuberculosis* H37Rv using a Glas-Col inhalation exposure system (Glas-Col Inc., Terre Haute, IN) and a fresh log-phase broth culture with the aim of implanting ca. 3.53 $\log_{10}$ CFU in the lungs of each mouse. Five mice were sacrificed the following day to determine the number of CFU implanted in the lungs. All animal procedures were approved by the Animal Care and Use Committee of Beijing Tuberculosis and Thoracic Tumor Research Institute (Beijing, China).

Table 1
Minimum inhibitory concentrations (MICs) of antimicrobial agents against *Mycobacterium tuberculosis* H37Rv.

Antimicrobial agent	MIC (mg/L)
INH	0.05
RIF	0.1
EMB	2.0
CFZ	0.24
LFX	0.5
LZD	0.5
PAS	0.125
AMK	1.0
PTO	2.0

INH, isoniazid; RIF, rifampicin; EMB, ethambutol; CFZ, clofazimine; LFX, levofloxacin; LZD, linezolid; PAS, *para*-aminosalicylic acid; AMK, amikacin; PTO, protionamide.

2.4. Chemotherapy

After infection, mice were randomized into eight treatment groups as described in Table 2. Group A was a negative control group in which mice were infected but untreated. Group H was a positive control group in which mice were treated with RIF + INH + PZA, the WHO's standard regimen. Mice in the remaining groups were treated with six different combination regimens. Treatment began 15 days after infection (on Day 0) in order to achieve a larger bacterial population and treatment was administered 5 days per week. For consistency, 4 weeks of treatment was considered as corresponding to 1 month. Drugs were administered at the following dosages (in mg/kg/day): LZD, 100; LFX, 200; PAS, 750; PTO, 100; CFZ, 20; AMK, 150; EMB, 100; RIF, 10; INH, 25; and PZA, 150. Based on the areas under the concentration–time curves, these dosages, which are similar to those used in previous experiments [14–16], were chosen as equipotent with the usual dosages administered to humans. All regimens were administered for 2 months. AMK was given by subcutaneous injection 5 days per week. The remaining drugs were administered by gavage.

2.5. Assessment of infection and treatment

To provide baseline values, five infected and untreated mice were killed on Days 1 and 15 after infection, respectively (Days −14 and 0 in relation to the initiation of treatment). Five mice per group were sacrificed after 1 month and 2 months of treatment for the determination of lung CFU counts.

The severity of infection and the effectiveness of the treatments were assessed by spleen weight, the number of CFU in the lungs, and the serum IFNγ and IL-10 levels. At Days −14 and 0 and after 1 month of treatment, the numbers of CFU in the lungs were determined by plating four serial 10-fold dilutions of homogenized suspensions onto OADC-enriched 7H11 agar medium (Becton Dickinson) and basic agar supplemented with 0.4% activated charcoal (Sigma) to reduce drug carry-over effects. After 2 months of treatment, the entire suspension prepared from each individual organ, which was supposed to contain few bacilli, was plated without dilution onto OADC-enriched 7H11 agar medium. Results for the cultures were recorded after incubation at 37 °C for 4 weeks. The bactericidal effect of the treatment was defined as a significant decrease in the mean number of CFU in the treated group compared with the pre-treatment value.