Disponible en ligne sur www.sciencedirect.com
ScienceDirect
Médecine et maladies infectieuses 37 (2007) 295–298
Médecine et maladies infectieuses
http://france.elsevier.com/direct/MEDMAL/

Letters to the editor

In vitro activity of ciprofloxacin, ofloxacin, levofloxacin, sparfloxacin and gatifloxacin against multidrug-resistant *Mycobacterium tuberculosis* in Rio de Janeiro, Brazil

Activité in vitro de la ciprofloxacine, ofloxacine, lévofloxacine, sparfloxacine et gatifloxacine sur huit souches de *Mycobacterium tuberculosis* multirésistantes tuberculosis à Rio de Janeiro, Brésil

Keywords: *Mycobacterium tuberculosis*; Fluoroquinolones; Multi drug resistance

Mots clés : *Mycobacterium tuberculosis* ; Fluoroquinolones ; Résistance aux antibiotiques

1. Introduction

Tuberculosis (TB) is an important public health problem worldwide due to the AIDS epidemic, multidrug-resistant (MDR) strains, and lack of new drugs on the market. This infectious disease kills between 2 and 3 million people each year, and it is estimated that there are 1 billion infected people with TB worldwide [1]. As a result, new drugs are urgently needed to fight this disease. In this context, fluoroquinolones are considered as a new class of anti-TB drugs because of their pharmacokinetic profile against Gram-negative and some Gram-positive bacteria, and because of their tolerance when administered either orally or IV [2]. The use of fluoroquinolones in clinical trials was first reported in 1985 by Tsukamura et al. [3], who used 6–8 months of ofloxacin (300 mg/day), combined with other drugs, to treat 19 patients presenting with drug resistant chronic TB. Currently, WHO approves fluoroquinolones as second-line agents to treat TB in patients with resistance or intolerance to first-line anti-TB agents [1]. However, the potential of fluoroquinolones as first-line agents is still under investigation.

The aim of this study was to determine the in vitro activity of fluoroquinolones against clinical strains of *Mycobacterium tuberculosis* identified in Rio de Janeiro, Brazil, with the highest number of new cases in this country. Accurate susceptibility data for the treatment of TB increases treatment success rates, while decreasing the spread of resistant strains and the rate of developing resistance to additional drugs. In this context, this study provides an important starting point by testing the susceptibility of *M. tuberculosis* to a class of drugs likely to be of increasing importance in treating TB infections.

2. Materials and methods

2.1. Strains studied

We studied 908 strains of *M. tuberculosis* identified in the city of Rio de Janeiro. These strains were maintained at –70 °C and subsequently re-suspended in Middlebrook 7H9 medium directly from solid medium and adjusted to a No. 1 McFarland standard. All strains were fully susceptible to rifampicin, isoniazide, streptomycin, ethambutol, and ethionamide (Sigma, St. Louis, USA), except for eight resistant strains. Three of these isolates were resistant only to izoniazid, one only to rifampicin, and one to rifampicin, isoniazide, and ethambutol. In addition, seven strains were resistant to rifampicin, isoniazide, and streptomycin, and one was resistant to rifampicin, isoniazide, streptomycin, and ethambutol. The eight highly-resistant strains were included in the present study.

2.2. Antibiotics tested

Rifampicin, isoniazide, streptomycin, ethambutol, and ethionamide were obtained from Sigma. Stock solutions of isoniazide, streptomycin, ethionamide, and ethambutol were prepared in de-ionized water, and rifampicin was prepared in dimethyl sulfoxide (DMSO). Ciprofloxacin, ofloxacin, levofloxacin, sparfloxacin, and gatifloxacin were supplied by Xiamen MChem Pharma Group Ltd. (Chine), and stock solutions were prepared in DMSO. Stock solutions were diluted in 7H9GC broth to two times the maximum desired final testing concentrations prior to their addition to micro plates.

2.3. Drug susceptibility testing

Susceptibility to rifampicin, isoniazide, streptomycin, ethambutol, and ethionamide was tested according to Canetti et al.'s [4] proportional method. The anti-mycobacterial activity of each fluoroquinolone was assessed on *M. tuberculosis* in 7H9 media, and the minimum inhibitory concentration (MIC) values were determined by a calorimetric, micro plate-based Alamar Bule assay (MABA) method as described by Franzblau et al. [5]. This methodology is simple, rapid, inexpensive, non-toxic, uses temperature-stable reagents, and shows good correlation with the proportional and BACTEC methods [6,7]. In the Franzblau methodology, 200 µl of sterile de-ionized water is added to all outer-perimeter wells of sterile 96 plates to minimize evaporation of the medium in the test wells during incubation. One hundred micro liters of 7H9GC broth were poured

Table 1
MICs (μg/ml) of selected fluoroquinolones against eight strains of MDR *M. tuberculosis* in Rio de Janeiro, Brazil
Tableau 1
Concentration minimale inhibitrice (μg/ml) de fluoroquinolones sur huit souches de *Mycobacterium tuberculosis* multirésistantes à Rio de Janeiro, Brésil.

Strain number	Ofloxacin (μg/ml)	Levofloxacin (μg/ml)	Ciprofloxacin (μg/ml)	Sparfloxacin (μg/ml)	Gatifloxacin (μg/ml)
H37rv	0.6	0.3	0.6	< 0.1	0.1
041	0.3	0.3	0.6	0.1	0.1
506	10	0.6	10	0.3	0.3
522	1.0	0.3	0.6	0.1	0.1
600	0.6	0.3	0.1	0.1	0.3
609	1.0	0.3	0.6	0.1	0.1
641	1.0	0.6	0.3	0.1	0.1
647	0.3	0.1	0.1	0.1	0.3
908	10	1.0	10	0.6	0.3

in the 96 plates and serial twofold dilutions of fluoroquinolones were carried out directly in the plate. The final drug concentrations tested were 0.1–10 μg/ml. Plates were covered and sealed and incubated at 37 °C for 5 days. After this time, we added 25 μl of a freshly prepared 1:1 mixture of 10 × Alamar Blue (Accumed International, Westlake, OH) reagent and 10% Tween 80 to the plates and incubated for an additional 24 hours. A blue color in the well was interpreted as absence of growth, and a pink color was considered to prove growth. The MIC was defined as the lowest drug concentration that prevented a color change from blue to pink.

3. Results and discussions

The activity of fluoroquinolones against *M. tuberculosis* is summarized in Table 1. All strains resistant to rifampicin, isoniazide, streptomycin, ethambutol, and ethionamide were susceptible to the fluoroquinolone assay. Sparfloxacin and gatifloxacin had the strongest in vitro activity (i.e. lowest MIC) on *M. tuberculosis* in six out of the eight studied strains. Furthermore, MICs for levofloxacin, sparfloxacin, and gatifloxacin were inferior to 1.0 μg/ml in the eight strains, and for ofloxacin and ciprofloxacin in six out of these eight strains.

4. Conclusion

The susceptibility to fluoroquinolones observed in strains resistant to traditional anti-TB drugs suggests that various fluoroquinolones may be effective therapeutic alternatives in infections caused by *M. tuberculosis*, including in patients with resistance or intolerance to first-line anti-TB therapy. In vitro and in vivo studies should be done to investigate this hypothesis.

References

[1] World Health Organization Tuberculosis Handbook. WHO Global Tuberculosis Programme. Geneva: The Organization; 1998.
[2] Da Silva AD, De Almeida MV, De Souza MVN, Couri MRC. Biological activity and synthetic methodologies for the preparation of fluoroquinolones, a class of potent antibacterial agents. Curr. Med. Chem. 2003;10:21–37.
[3] Tsukamura M, Nakamura E, Yoshii S, Amano H. Therapeutic effect of a new antibacterial substance ofloxacin (DL8280) on pulmonary tuberculosis. Am Rev Respir Dis 1985;131:352–6.
[4] Canetti J, Rist E, Grosset R. La méthode des proportions. Review of Tuberculosis Pneumology 1963;27:217–72.
[5] Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A, et al. Rapid low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the Microplate Alamar Blue Assay. J Clin Microbiol 1998;36:362–6.
[6] Vanitha JD, Paramasivan CN. Evaluation of microplate Alamar Blue Assay for drug susceptibility testing of *Mycobacterium avium* complex isolates. Mycobacteriology 2004;49:179–82.
[7] Reis RS, Neves Jr. I, Lourenço SLS, Fonseca LS, Lourenço MCS. Comparison of flow cytometric and Alamar Blue Tests with the proportional method for testing susceptibility of *Mycobacterium tuberculosis* to rifampin and isoniazid. J Clin Microbiol 2004;42:2247–8.

M.C.S. Lourenço
I.N. Junior
IPEC-Instituto de Pesquisas Clínicas Evandro Chagas, Instituto de Bacteriologia, Rio de Janeiro-RJ, Brazil

M.V.N. De Souza*
Instituto de Tecnologia em Fármacos-Far-Manguinhos Rua Sizenando Nabuco, 100, Manguinhos, 21041-250 Rio de Janeiro-RJ, Brazil
E-mail address: marcos_souza@far.fiocruz.br
(M.V.N. De Souza).

Received 18 November 2006; accepted 7 March 2007

Available online 24 April 2007

*Corresponding author.

doi:10.1016/j.medmal.2007.03.011