

Evaluation of Moxifloxacin Activity *In Vitro* Against *Mycobacterium tuberculosis*, Including Resistant and Multidrug-Resistant Strains

E. TORTOLI¹ - D. DIONISIO² - C. FABBRI²

¹ Centro Regionale di Riferimento per la Diagnostica dei Micobatteri, Laboratorio di Microbiologia e Virologia, Ospedale di Careggi, Florence, Italy; ² Divisione di Malattie Infettive, Spedali Riuniti, Pistoia, Italy.

Corresponding author: Enrico Tortoli, Centro Regionale di Riferimento per la Diagnostica dei Micobatteri, Laboratorio di Microbiologia e Virologia, Ospedale di Careggi, viale Morgagni 85, 50134 Firenze, Italy. Phone +39055.4279199. Fax +39055.4279010. E-mail: e.tortoli@libero.it

Summary

The new quinolone moxifloxacin was tested against 86 strains of *Mycobacterium tuberculosis* including 13 resistant and 4 multiresistant strains. The antimicrobial susceptibility was tested, in parallel, using two different liquid media, the radiometric Bactec 12B and the Mycobacteria Growth Indicator Tube (Becton Dickinson, USA). All strains but two were susceptible at 0.5 µg/ml of moxifloxacin; for the remaining two strains, both Multidrugresistant, the minimal inhibitory concentrations (MIC) were =2 and >4 µg/ml respectively. Our data confirm the high antitubercular *in vitro* activity of moxifloxacin.

Key words: Susceptibility testing, resistance, multidrug-resistance, moxifloxacin, *M. tuberculosis*

INTRODUCTION

Tuberculosis is far from being a disease of the past and the specter of the "white plague" is still at large, not only in developing countries, but even in major cities of the industrialized world¹. The drug resistance of *Mycobacterium tuberculosis*, and in particular the multidrug resistance² are a major concern today, all the more because the number of antitubercular drugs is limited and the research for new molecules has almost been completely abandoned.

In recent decades the only new antimycobacterial drugs are molecules developed against microorganisms other than mycobacteria. Obvious examples are quinolones³ and, with regard to nontuberculous mycobacteria, macrolides⁴.

The recent development of a highly active quinolone, moxifloxacin⁵, led us to investigate its activity against recently isolated *M. tuberculosis* strains, including some which are resistant to one or more drugs.

MATERIAL AND METHODS

The 86 strains on which the susceptibility testing was performed were isolated in the mycobacteriology laboratory routine from clinical specimens obtained from different patients. The identification of the cultures as belonging to the species *Mycobacterium tuberculosis* was obtained by combining DNA probe identification (INNO LiPA MYCOBACTERIA, Belgium), niacin accumulation and nitrate reduction tests⁶. The majority of such strains were susceptible to the first-line antituberculosis drugs (ethambutol, isoniazid, pyrazinamide, rifampicin and streptomycin), 17 of them, however, were resistant to one or more drugs.

The susceptibility testing was performed in liquid medium using the radiometric method (Bactec, Becton Dickinson, USA)⁷. With this approach, which is considered the gold standard, the resistance of *M. tuberculosis* is disclosed by the liberation of

radio-labeled CO₂ operated by metabolically active mycobacteria even in the presence of the drug. The susceptibility was also determined with the newly developed Mycobacteria Growth Indicator Tube medium (MGIT, Becton Dickinson, USA) ⁸ in which the bacterial growth (i.e. the resistance) is revealed by the fluorescence development consequent to the oxygen consumption. In both media the testing was performed in a qualitative manner. Universally agreed cut-offs exist for the first-line antitubercular drugs only; however, as 1 µg/ml is generally adopted for ciprofloxacin and ofloxacin, the most used quinolones in tuberculosis therapy, we decided to choose a lower cut-off of moxifloxacin by halving the concentration to 0.5 µg/ml as we hypothesized that its activity would be greater.

In both assays the media were inoculated, conforming to the principle of proportions. In short, in the Bactec method, 100 µl of a radiometric culture with a growth index (GI) between 500 and 800 was used to directly inoculate the vial containing 0.5 µg/ml of moxifloxacin while 100 µl of its 1/100 dilution were added to the antibiotic-free control vial. The vials were read daily with the Bactec 460TB instrument until the achievement, by the control vial, of a growth index >30; at that moment the difference (ΔGI) was calculated between the actual GI value and the one of the previous day, both for the control and the drug-containing vial. A ΔGI, in the moxifloxacin-containing vial, greater or equal to the one of the control, was interpreted as resistance.

In the MGIT method a broth culture scored positive by the MGIT 960 instrument in which it had been incubated at least one, but no more than two days before, was used for the inoculum. Two tubes had been added with 0.8 ml of proper supplement (Becton Dickinson) and, again, the one containing 0.5 µg/ml of moxifloxacin was directly inoculated (0.5 ml) while for the control 0.5 ml of a 1/100 dilution was used. The tubes were placed in proper 2-tube MGIT racks (with the control absolutely preceding the moxifloxacin-

containing broth) and incubated in the MGIT instrument until the conclusion of the test. The final result was automatically signaled when the fluorescence of the control was equal to 400. Every value of the moxifloxacin-containing tube >400 was interpreted as resistance.

The purity of mycobacterial cultures was checked by dropping a few microliters of the inoculum broth in blood agar and Middlebrook 7H11 plates which were incubated and read daily to detect the presence of possible contaminants. The drug-containing broth cultures showing resistance were checked for purity as well.

In the cases in which resistance was obtained with one or both methods the test was repeated using also twofold moxifloxacin concentrations from 0.5 to 4 µg/ml.

Moxifloxacin (BAY 12-8039) was provided by Bayer S.p.A. (Italy).

RESULTS AND DISCUSSION

Eighty-four of the strains tested turned out to be susceptible to moxifloxacin according to the Bactec method, and 80 with MGIT. The repetition of tests on the strains presenting resistance with one or both methods confirmed, in all the cases in which resistance was reported by MGIT only, the susceptible result obtained with Bactec.

In one of the two confirmed cases of moxifloxacin resistance the minimal inhibitory concentration (MIC) was 2 µg/ml; in the other it exceeded the highest concentration tested (4 µg/ml). Both strains were multidrug-resistant; the one with the lower MIC was resistant to isoniazid and rifampin, and the other to all the first line drugs (Table 1). Unfortunately no information was obtained about possible previous treatments with quinolones in the two patients from which the moxifloxacin-resistant strains were grown.

TABLE 1.- Susceptibility results of the *M. tuberculosis* strains presenting resistance to at least one drug. Sixty-nine strains were fully susceptible.

N. strains	Ethambutol	Isoniazid	Pyrazinamide	Rifampin	Streptomycin	Moxifloxacin
10 ^a	S	R	S	S	S	S
1 ^a	S	S	S	R	S	S
1 ^a	S	S	S	S	R	S
1 ^b	S	R	S	S	R	S
1 ^c	R	R	S	R	S	S
1 ^c	S	R	S	R	S	R (MIC > 4)
1 ^c	S	R	S	R	R	S
1 ^c	R	R	R	R	R	R (MIC = 2)

^a monoresistance; ^b double resistance; ^c multiresistance

Twelve of the strains susceptible to 0.5 µg/ml of moxifloxacin were mono-resistant, and 3 were resistant to two or more drugs (Table 1).

The percentage of resistance to moxifloxacin (2.3%) in the strains tested here exceeded that of pyrazinamide (1.2%), although resistance to other drugs, except for ethambutol, which was identical, was higher.

In the work of Rodriguez et al.⁹, the only one involving a high number of strains, a MIC₉₀ for moxifloxacin of <0.5 µg/ml and 2% resistance are reported. The other studies, carried out with limited numbers of strains, show MIC_{90s} ranging from 0.25 µg/ml, on Lowenstein-Jensen medium¹⁰, to 0.5 µg/ml on Middlebrook 7H11 medium¹¹ and in liquid non-radiometric medium¹². Such results are in agreement with ours in which 98% of the strains have a MIC ≤0.5 µg/ml. Furthermore our study is the first in which the radiometric approach was used.

The use of MGIT in parallel with the reference radiometric method seems to support the possible use of this system too, provided the cases in which resistance is detected are carefully confirmed. The initial excess of resistance estimation obtained with the MGIT system is probably due to the high risk of contamination characterizing the method as hypothesized in a previous study⁸.

According to the literature data, the ability of moxifloxacin to penetrate into the tissues and to accumulate in the macrophages, along with its long half-life, its high plasma concentration and high activity at concentrations as low as 0.5 µg/ml, makes this novel quinolone a promising addition to the narrow number of drugs active against *M. tuberculosis*.

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