

In vitro activity of ciprofloxacin, ofloxacin and levofloxacin against *Mycobacterium tuberculosis*

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BACKGROUND: The increasing incidence of drug-resistant *Mycobacterium tuberculosis* necessitates therapeutic alternatives. The fluoroquinolones fulfill most of the criteria for an ideal class of antimycobacterial drugs. The aim of the present study was to determine in vitro activities of ciprofloxacin, ofloxacin, and levofloxacin against *M. tuberculosis* strains.

METHODS: Susceptibility to four antituberculous drugs used in first-line treatment of tuberculosis was tested in 100 strains isolated from clinical samples. Nineteen strains (19%) were resistant to at least one of the four antituberculous drugs and 13 were multidrug resistant. The in vitro antimycobacterial activity of ciprofloxacin, ofloxacin, and levofloxacin was then determined against 100 *M. tuberculosis* strains using standard agar proportion dilution method.

RESULTS: Ciprofloxacin, ofloxacin, and levofloxacin were active against all tested strains of *M. tuberculosis* in vitro.

CONCLUSIONS: Ciprofloxacin, ofloxacin, and levofloxacin have relatively potent in vitro activity against *M. tuberculosis*. Further in vivo studies are needed to determine the role of these compounds in the treatment of tuberculosis, but use should be limited to special circumstances rather than first-line treatment.

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Diseases caused by mycobacteria are often difficult to treat and require regimens containing several drugs administered over a prolonged period. Relatively few drugs are effective against mycobacteria, especially multidrug-resistant *Mycobacterium tuberculosis*. Hence, development of new classes of antimycobacterial agents is needed. The fluoroquinolones would appear to fulfill most of the criteria for an ideal class of antimycobacterial drugs. The in vitro activity of fluoroquinolones against mycobacteria and the efficacy of these drugs in murine models of mycobacterial infection have been documented in various studies and reviews.¹⁻³ Some fluoroquinolones are currently used to treat mycobacterial infections.⁴ The mechanism of action of the quinolone antibacterial agents is thought to result from the combination of their abilities to penetrate into bacterial cells and inhibit DNA gyrase, an essential bacterial enzyme that maintains the superhelical twist of DNA.⁵⁻⁶

In the present study, the in vitro antimycobacterial activity of ciprofloxacin, ofloxacin, and levofloxacin was determined against 100 *M. tuberculosis* isolates.

Methods

One hundred strains of *M. tuberculosis* isolated from various clinical samples in the Clinical Microbiology Laboratory of Celal Bayar

University Hospital were included in this study. All strains were isolated by culturing on Löwenstein-Jensen slants. Organisms were identified to the species levels by standard methods and the BACTEC NAP test.⁷ *M. tuberculosis* ATCC 27294, ATCC 35838, ATCC 35825, ATCC 35837 were used for internal quality control. Four of the six anti-tuberculosis drugs used in first-line treatment-isoniazid (INH), rifampicin (RIF), streptomycin (SM), and ethambutol (ETB) were tested in the study. These drugs were chosen because they have been, and continue to be widely used throughout the world, and resistance can be reliably measured by standardized techniques that have been studied for many years. Given the difficulties in standardizing susceptibility testing for pyrazinamide, this drug is not routinely included in the panel of antituberculous drugs tested for surveillance purposes.⁸ Testing of susceptibility to first-line antituberculous drugs was performed by the agar proportion method (Table 1). Of the strains tested, 19 (19%) were resistant to at least one first-line antituberculosis drug (6 and 13 multidrug-resistant strains) while the rest were fully susceptible (monoresistant) (Table 2).

The definitions of resistance were those recommended by the World Health Organisation (WHO) and International Union Against Tuberculosis and Lung Disease (IUATLD).⁸ Any drug resistance was defined as resistance to one or more first line drugs, whereas multidrug resistance was defined as resistance to both isoniazid and rifampicin with or without resistance to other agents. Ciprofloxacin, ofloxacin and levofloxacin were obtained from the Sigma Chemical Company. The following drugs and concentrations were included in agar proportion susceptibility tests: ciprofloxacin 2 mg/L, ofloxacin 2 mg/L, levofloxacin 2 mg/L. Antibacterial activity was determined by an agar dilution technique using Middlebrook's 7H10 agar. Standard Middlebrook 7H10 agar and oleic-albumin-dextrose-catalase (OADC) enrichment were used to prepare all drug-containing media.⁷ Stock solutions of the agents were prepared on the day of testing according to the recommendations of the manufacturers.

Standard agar proportion dilution methods were used in this study. Colonies from a Löwenstein-Jensen tube were homogenised in phosphate buffered saline (pH 7.0) to achieve turbidity equal to a McFarland 1.0 standard, corresponding to approximately 10⁹ CFU/mL. 7H10 agar medium (Difco) was prepared from a dehydrated base as recommended by the manufacturer. After the agar was

autoclaved, oleic acid-albumin-dextrose-catalase supplement (Becton-Dickinson) and fluoroquinolones were added at 50°C to 56°C by doubling dilutions to yield final concentrations of ciprofloxacin 2 mg/L, ofloxacin 2 mg/L, and levofloxacin 2 mg/L. Five mL of each concentration of antimycobacterial-containing medium was dispensed into plastic quadrant petri dishes. As a growth control, one quadrant in each plate was filled with 7H10 agar medium with no drug. An inoculum of each isolate was prepared in Middlebrook 7H9 broth, and the absorbance was adjusted until it was equivalent to that of a McFarland No. 1 standard. Final suspensions were performed by adding phosphate buffered saline to prepare 10⁻² and 10⁻⁴ dilutions of the standardized suspensions. Upon solidification of the medium, the plates received 0.1 mL of the dilutions by inoculation of 3 drops at different points on each quadrant of the agar plates. The inoculated plates were then incubated at 37°C for 3 weeks. In the agar proportion dilution methods, an isolate was classified as susceptible to a drug if the number of colonies that grew on the drug-containing plate was <1% of the number of colonies that grew on a control plate without drug, partially resistant if the number was between 1% and 10%, and resistant if the number was >10%. In cases where two drug concentrations were tested in the agar proportion dilution method, an isolate was classified as partially resistant if it exhibited resistance at the lower concentration but was susceptible at the higher of the two concentrations tested.

Results

Ciprofloxacin, ofloxacin, and levofloxacin were active against all tested strains in vitro.

Discussion

Tuberculosis (TB), one of the oldest known diseases in the world, has been recognised for 116 years and effective chemotherapeutic regimens have existed for 54 years. Though it has remained a challenge for developing countries, industrialized countries have partly controlled TB. However, due partly to AIDS and mass population movements, TB has again emerged as a serious global public health issue.⁹ The emergence of drug-resistant tuberculosis (DR-TB), especially multidrug-resistant tuberculosis (MDR-TB), poses a major threat to the control and prevention of TB.¹⁰⁻¹² It is known that the rate of primary resistance is approximately 5% or less in effective national programs, and 15% or more in newly implemented programs.¹³ Because of this high degree

of drug resistance, development of new drugs having strong anti-autoTB activity is urgently needed.

A number of fluoroquinolones have been or are being developed.¹⁴⁻¹⁶ Among the quinolones, ciprofloxacin, ofloxacin, and levofloxacin may be promising for the treatment of TB, particularly MDR-TB. The incidence of mycobacterial resistance to fluoroquinolones is relatively low at present, and there are no reports of cross-resistance or antagonism with other classes of antimycobacterial drugs. Fluoroquinolones can be administered orally, with good absorption and favorable pharmacokinetics, including extremely efficient penetration into tissues and host macrophages. Moreover, the incidence and severity of adverse effects are generally low for the fluoroquinolones. Thus, fluoroquinolones may be used for long-term therapy of tuberculosis patients, especially those with HIV infection, in combination with other antimycobacterial drugs.¹⁷

The cut-off points of these compounds against *M. tuberculosis* have not been established clearly, although it has been suggested that for ciprofloxacin and ofloxacin, 2 mg/L would be suitable; this was confirmed in a recent multicentric study.¹⁸ However, Gross et al.¹⁹ reported that the MIC of strains sensitive to ciprofloxacin was between 0.25 mg/L and 3 mg/L. These discrepancies and the emergence of strains with a high MIC against these compounds make it necessary to standardise the in vitro evaluation of the activity of these compounds against *M. tuberculosis*.¹⁸

In this study we compared the in vitro antimycobacterial activity of a new quinolone, levofloxacin, with that of two of the older fluoroquinolones (ciprofloxacin and ofloxacin) against strains of *M. tuberculosis* isolated in Manisa, Turkey. We found all three fluoroquinolones to be bactericidal against *M. tuberculosis* at a concentration of 2 mg/L. Rodriguez et al.¹⁸ found that the MIC₅₀ for ciprofloxacin and ofloxacin was 1 mg/L, while it was less than 0.5 mg/L for levofloxacin and moxifloxacin. They reported that the MIC₉₀ for ciprofloxacin was 4 mg/L, for ofloxacin 2 mg/L, and for levofloxacin and moxifloxacin 1 mg/L. La Bombardi et al.²⁰ found that 4% of their strains had a MIC to ciprofloxacin greater than 2 mg/L and Vacher et al.²¹ found an MIC₉₀ of 1 mg/L for ciprofloxacin and ofloxacin, with none of their 33 strains having an MIC above 2 mg/L. In another investigation, MICs of 14 first- and second-line antituberculous drugs against drug-susceptible and drug-resistant *M. tuberculosis* isolates were determined radiometrically by Rastogi et al.²² Their

Table 1. Critical concentrations used for the agar proportion method on Middlebrook 7H10 agar.

Agent	Critical concentration (mg/L)
Isoniazid	0.2
Rifampin	1.0
Streptomycin	2.0
Ethambutol	5.0
Ciprofloxacin	2.0
Ofloxacin	2.0
Levofloxacin	2.0

Table 2. Resistance distribution of isolates resistant to one or more first-line antituberculosis drugs.

Antituberculosis Drug	Resistant isolates (n)	Distribution in resistant population (%)	Distribution in total population (%)
Single drug			
SM	6	31	6
Two drugs			
INH+RIF	1	5	1
INH+SM	1	5	1
INH+ETB	3	16	3
Three drugs			
INH+RIF+SM	1	5	1
RIF+SM+ETB	1	5	1
Four drugs			
INH+RIF+SM+ETB	6	31	6

SM=streptomycin, INH=isoniazid, RIF=rifampicin, ETB=ethambutol

data showed that the activity of the second-line drugs remained unaltered in case of MDR-TB isolates usually resistant to routine first-line drugs only; e.g., the three quinolone drugs were active against all ten strains, with the order of activity sparfloxacin > ofloxacin > ciprofloxacin. In the other study, Hoffner et al.²³ investigated the in vitro activities of seven fluoroquinolone and macrolide compounds against 23 clinical isolates of *M. tuberculosis*, including 17 multi-drug resistant strains. Sparfloxacin was the most active fluoroquinolone, with MICs ≤ 1 mg/L for all tested strains, followed by levofloxacin and ciprofloxacin. Travofloxacin had no inhibitory activity at the concentration tested. Our data are similar to these results. We found 13 MDR-TB strains and all were susceptible to ciprofloxacin, ofloxacin and levofloxacin.

The in vitro activities of eight new quinolones (A-56620, amifloxacin, difloxacin, CI-934, enoxacin, irloxacin, pefloxacin, temafloxacin) and two ref-

erence fluoroquinolones (ciprofloxacin, ofloxacin) against 147 mycobacterial strains, belonging to seven different species, were compared by Caekenberghe.²⁴ Temafloxacin, ofloxacin and ciprofloxacin were the most active compounds. They only showed consistent activity against *M. tuberculosis*, *M. malmoense* and *M. fortuitum*, but not against *M. chelonae*, *M. avium*, *M. intracellulare* and *M. scrofulaceum*.

In conclusion, we found that ciprofloxacin, ofloxacin and levofloxacin have relatively potent in vitro activity against *M. tuberculosis*. An in vivo evaluation of the antimycobacterial activity of these compounds is needed. Nevertheless, such valuable drugs should not be considered for primary treatment as first-line agents and should be saved for special treatment circumstances.

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