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Comparison of the bactericidal activity of various fluoroquinolones against *Mycobacterium tuberculosis* in an *in vitro* experimental model

Rosa Cremades, Juan Carlos Rodríguez*, Eduardo García-Pachón, Antonio Galiana, Montserrat Ruiz-García, Pilar López and Gloria Royo

Hospital General Universitario de Elche, Universidad Miguel Hernández, Elche, Alicante, Spain

*Corresponding author. Tel: +34-966-616131; Fax: +34-966-616123; E-mail: rodriguez_juadia@gva.es

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Objectives: To compare the bactericidal activity of various fluoroquinolones against *Mycobacterium tuberculosis* in the latent and exponential growth phases.

Methods: Ciprofloxacin, levofloxacin and moxifloxacin were tested against 16 *M. tuberculosis* clinical isolates (4 resistant and 12 susceptible to fluoroquinolones) from Elche, Spain, isolated between 1992 and 2009. To study bactericidal activity, an inoculum of approximately 10^5 cfu of each isolate was cultured in Middlebrook 7H9 broth. The broth was previously acidified to pH 4.6 to obtain the microorganism in the stationary phase. Cultures with different concentrations (0.1 to 50 mg/L) of antibiotic and antibiotic-free controls were incubated for 48 h then plated onto Middlebrook 7H11 to detect bacterial killing. In all stages of the process the *M. tuberculosis* strain ATCC 41323 was included as a quality control to ensure reproducible results.

Results: Moxifloxacin and levofloxacin were found to exhibit bactericidal activity at lower concentrations and against more strains in both the latent and the exponential growth phases compared with ciprofloxacin. The bactericidal activity of moxifloxacin was greater than that of levofloxacin against microorganisms in the exponential growth phase, but the opposite was true in the latent phase.

Conclusions: Our data confirm the usefulness of moxifloxacin in the treatment of tuberculosis and suggest that levofloxacin may be used as an alternative drug in the treatment of latent tuberculosis when it is not possible to use isoniazid. Based on the results presented, ciprofloxacin appears to be a poor choice.

Keywords: *in vitro* susceptibility, moxifloxacin, ciprofloxacin, levofloxacin, latent growth phase

Introduction

Tuberculosis is a serious public health problem worldwide, especially when the microorganism is resistant to isoniazid and rifampicin. In such cases, fluoroquinolones are one of the most interesting therapeutic alternatives. In order to determine which of this family of compounds is the most useful in the treatment of this disease, we developed an *in vitro* experimental model to compare bactericidal activity against two groups of microorganisms present in tuberculosis; those in the exponential growth phase and those in the latent phase.

Materials and methods

Strains

Sixteen *Mycobacterium tuberculosis* clinical isolates [4 resistant (MIC ≥4 mg/L) and 12 susceptible (MIC ≤1 mg/L) to fluoroquinolones according to CLSI criteria] from Elche, on the Mediterranean coast in the southeast of Spain, were used in this study. They were isolated between 1992 and 2009.

Antibiotics

Ciprofloxacin was obtained from Bayer Pharma (Barcelona, Spain), levofloxacin from Aventis Pharma (Barcelona, Spain) and moxifloxacin from Bayer Pharma (Barcelona, Spain).

Study of bactericidal activity

An inoculum of approximately 10^5 cfu of each isolate was cultured in 10 mL of Middlebrook 7H9 broth (Difco, New York, USA) without shaking. The broth was previously acidified to pH 4.6 with the addition of hydrochloric acid to obtain the microorganism in the stationary phase, as described previously.[1] In addition, quantitative studies were done to confirm the ability of this system to achieve the stationary growth phase in these bacteria.

Table 1. Comparison of the bactericidal activity of various fluoroquinolones against *M. tuberculosis* strains: percentages of bactericidal activity at pH 7/pH 4.6

	Susceptible			Resistant		
Antibiotic concentration (mg/L)	MXF	CIP	LVX	MXF	CIP	LVX
Control	—	—	—	—	—	—
0.1	—	—	—	—	—	—
0.5	—	—	—	—	—	—
1	—	—	8.3/—	—	—	—
2	25/—	—	8.3/—	25/—	—	—
4	50/—	—	16.6/—	25/—	—	—
8	66.6/41.6	—/16.6	33.3/16.6	25/—	—	—
16	83.3/58.3	16.7/16.6	75/83.3	25/—	25/—	25/—
50	100/100	50/16.6	83.3/100	50/25	25/—	25/—

MXF, moxifloxacin; CIP, ciprofloxacin; LVX, levofloxacin.
It was felt that the drugs had sterilizing activity if no visible growth was detected after 30 days of incubation of the subcultures.

Table 2. Pharmacokinetic parameters of the drugs studied

	Maximum serum concentration md (mg/L)	Maximum serum concentration md/MBC_{75}	AUC md	AUC md/MBC_{75}	Lung epithelium (mg/L)	Lung epithelium/MBC_{75}	Alveolar macrophage (mg/L)	Alveolar macrophage/MBC_{75}
CIP	3.61–4.2	—	12.13–29.1	—	1.9	—	34.9	—
LVX	8.6–11.8	0.54–0.73	90.7–118	5.67–7.38	9.9–22.1	0.62–1.38	28.5–105.1	1.78–6.57
MXF	3.98–4.5	0.25–0.28	39–48	2.44–3	11.7–20.7	0.73–1.29	56.7–123.3	3.54–7.7

CIP, ciprofloxacin; LVX, levofloxacin; MXF, moxifloxacin; md, after multiple dosing; MBC_{75}, MBC for 75% of the strains (mg/L); AUC, total area under the serum curve (mg·h/L).

Cultures were maintained without antibiotic (control) and with antibiotics at different concentrations (0.1, 0.5, 1, 2, 4, 8, 16 and 50 mg/L).

In order to determine the bactericidal activity of the antibiotics, after incubation for 48 h at 37°C, subcultures (100 μL of liquid medium) were performed on plates of Middlebrook 7H11 (Difco, New York, USA) to establish that the liquid cultures were negative (total eradication of all microorganisms).

Quality control

In all stages of the process, the *M. tuberculosis* strain ATCC 41323 was included as a quality control to ensure reproducible results.

Results

Comparison of the activity of the three drugs showed that moxifloxacin and levofloxacin were more effective than ciprofloxacin against susceptible microorganisms in the exponential growth phase. At 16 mg/L, moxifloxacin and levofloxacin exhibited bactericidal activity against at least 75% of the strains, whereas ciprofloxacin did not, even at a higher concentration (50 mg/L). See Table 1.

Comparing the activity against microorganisms in the stationary phase, only levofloxacin was seen to exhibit bactericidal activity against at least 75% of the strains at 16 mg/L; moxifloxacin exhibited this activity at 50 mg/L, whereas ciprofloxacin did not do so in our experimental model. The *in vitro* activity of the drugs studied against resistant strains was low, even at very high concentrations, and only moxifloxacin exhibited activity against 50% of the resistant strains in exponential growth at 50 mg/L. See Table 1.

When these data were compared with the pharmacological parameters of the antimicrobials, ciprofloxacin was seen to be the drug that reached the lowest levels, both in serum and in pulmonary tissue. This, together with its lower *in vitro* activity, confirms that it is less useful as an antituberculostatic drug. Comparing the levels of levofloxacin and moxifloxacin, levofloxacin was seen to reach a higher serum concentration and greater AUC, whereas moxifloxacin reached higher levels in pulmonary tissue.[2] The quotients of pharmacokinetic parameters and drug concentrations that exhibited bactericidal activity against at least 75% of susceptible strains are shown in Table 2.

Discussion

Current treatment for tuberculosis resistant to traditional drugs is very problematic due to the scarcity of highly effective alternative drugs. This makes prolonged treatment necessary, thus favouring lack of adherence to treatment, together with

therapeutic failures and the emergence of new resistance. Therefore, one of the most important objectives in the control of this disease in the future is to develop shorter, more effective therapeutic regimens. In this respect, the use of drugs with bactericidal activity is crucial.[3]

Furthermore, the metabolic situation of *M. tuberculosis* in the human body varies; it may be in the exponential growth or stationary phase depending on the characteristics of the process (low oxygen tension, quorum sensing and old age of microorganisms). Thus, to be effective, chemotherapy should be active against all the bacterial populations present in the lesion.[4]

Our model confirms the limited antituberculosis activity of ciprofloxacin and shows that moxifloxacin is the most active fluoroquinolone against microorganisms in the exponential phase. However, our model also shows that levofloxacin is more active against microorganisms in the stationary phase, which confirms the findings of a previous study published by our group.[5] This fact should be analysed in depth since the effectiveness of therapy is associated with a greater elimination of microorganisms in the stationary phase. It has also been suggested that the fluoroquinolones could be used in the treatment of contacts in cases of resistant tuberculosis.[6]

Another finding of our model is that the bactericidal activity of the drugs studied is related to the concentration used. This indicates that the usefulness of different drugs should be evaluated as a function of their pharmacokinetic parameters in order to achieve the necessary levels at the site of infection. It should be noted that the AUC of levofloxacin is greater than that of moxifloxacin, which is related to greater tissue distribution of the drug. This supports the possibility of using levofloxacin to treat microorganisms in the stationary phase, although the pharmacological parameters of moxifloxacin are higher in the lungs. This should be taken into account when treating the latent disease of HIV patients, since isoniazid has been reported to have important limitations.[7]

The optimal ratio between pharmacological parameters and *in vitro* drug activity is not known for the fluoroquinolones, but the quotient of AUC and drug concentration that exhibits activity against at least 75% of susceptible strains in exponential growth is 7.3 for levofloxacin and 3 for moxifloxacin. In the case of pyrazinamide, the quotient AUC/MIC has been reported to be 209,[8] which indicates that higher doses of these compounds should be used to treat tuberculosis.

It is important to note that the use of drugs with bactericidal activity limits the emergence of drug-resistant microorganisms. As demonstrated by Lipsitch and Levin,[9] the relative rates of killing are more important than mutation rates in determining the order in which resistant mutants appear. Our model shows that moxifloxacin is the drug that exhibits the greatest bactericidal activity against microorganisms in the exponential phase, and these are the ones that are responsible for the emergence of resistant mutants. This confirms the usefulness of this drug for treating tuberculosis and confirms the need to use high doses to achieve bactericidal activity. This has been reported previously in Monte Carlo simulations, which showed that, for patients taking moxifloxacin doses of 400, 600 or 800 mg/day, the calculated target attainment rates to suppress drug resistance were 59%, 86% and 93%, respectively.[10] Our model shows the low bactericidal activity of moxifloxacin against fluoroquinolone-resistant strains, even at very high concentrations. Thus, it is necessary to determine the *in vitro* susceptibility of this compound before using it to treat patients.

This study shows that moxifloxacin exhibits bactericidal activity *in vitro* against susceptible microorganisms in the exponential phase, thus confirming its usefulness for treating tuberculosis. On the other hand, levofloxacin was seen to be more active against microorganisms in the stationary phase, which suggests it may be used to treat latent tuberculosis when it is not possible to use isoniazid.

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Transparency declarations

None to declare.

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