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In Vitro Activities of Ofloxacin, Levofloxacin and Norfloxacin Against Multi-Drug Resistant Mycobacterium tuberculosis Strains (*)

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SUMMARY

As the fluoroquinolones are novel anti-tuberculosis drugs to be used in multi-drug resistant tuberculosis (MDR-tb), minimal inhibitory concentrations (MIC's) of ofloxacin , levofloxacin and norfloxacin were investigated by radiometric proporsion method in 20 MDR Mycobacterium tuberculosis (M.tuberculosis) strains. MIC_{50} and MIC_{90} values of ofloxacin, levofloxacin and norfloxacin were found to be as 1µg/ml , 0.5 µg/ml , 5 µg/ml and 2µg/ml,

1 µg/ml, 10 µg/ml respectively. MIC values of levofloxacin were more lower than ofloxacin but the highest MIC values were obtained in norfloxacin. As a result, we concluded that ofloxacin and levofloxacin can be used as alternative drugs in the treatment of MDR-tb caused by the strains isolated from our laboratory.
Key words: M.tuberculosis , multi-drug resistant tuberculosis , fluoroquinolones

ÖZET

Ofloksasin, Levofloksasin ve Norfloksasinin Çoğul İlaca Dirençli M.tuberculosis Suşlarına Karşı in vitro Etkinliği

Fluorokinolonlar çoğul ilaca dirençli suşlar tarafından oluşturulan tüberkülozun tedavisinde yer aldıklarından , ofloksasin , levofloksasin ve norfloksasinin minimal inhibitor konsantrasyonları (MIC's) , 20 çoğul ilaca dirençli M.tuberculosis suşuna karşı radyometrik proporsiyon yöntemiyle araştırıldı.Ofloksasin , levofloksasin ve norfloksasinin MIC_{50} ve MIC_{90} değerleri sırasıyla 1-0.5-5 µg/ml ve 2-1-10 µg/ml olarak belirlendi.Levofloksasinin MIC değerleri ofloksasinden daha düşük bulundu ve en yüksek MIC değerleri norfloksasin için elde edildi. Sonuç olarak laboratuvarımızdan izole edilen çoğul ilaca dirençli M.tuberculosis suşlarının oluşturduğu tüberkülozun tedavisinde ofloksasin ve levofloksasinin alternative ilaç olabileceği belirlendi.

Anahtar kelimeler : M.tuberculosis , çoğul ilaca dirençli tüberküloz, fluorokinolonlar

INTRODUCTION

Tuberculosis remains one of the main problem worldwide and the emergence of MDR-M.tuberculosis strains has become a major concern.The decreased activity of first line drugs,especially to isoniazid and rifampin causes some problems in the treatment of MDR-tb. Compared with the other infections,drugs clinically active to M.tuberculosis relatively more fewer (1). Because of this reason alternative therapies are required urgently and in vitro activities of drugs should be tested in clinical laboratories in this meaning.

Several quinolones were demonstrated to be active in vitro and in vivo against mycobacterial strains and are increasingly being used in combination with other agents to threat tuberculosis (2,3,4,5,6).

Fluoroquinolones are synthetic anti-bacterial agents derived from the first pyrridone-beta-carboxylic derivative,nalidixic acid. They have a characteristic fluorine atom at position 6 and aryl substituent at position 7 of the quinoline or 1,8 naphthyridone ring (1).

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Ofloxacin,a pyridonecarboxylic acid derivative of nalidixic acid,has an asymmetric center at the C-3 position of the oxazine ring and this fluoroquinolone exists as a racemic mixture (7). Levofloxacin is the pure (-)-(S)-enantiomer of the racemic drug substance ofloxacin and it has become available for therapy in United States and in Italy (5).

Quinolones inhibit bacterial type II topoisomerase , DNA gyrase and topoisomerase IV. DNA gyrase is composed of two A and two B subunits,encoded by gyrA and gyrB respectively (8). Altought mutations in gyrB gene have not been reported yet for M.tuberculosis strains,mutations in gyrA gene have been associated with high-level resistance to fluoroquinolones(9). Due to the mutations in gyrA gene MIC values of fluoroquinolones were increased 4- to 16- fold (single missence mutations) or 32-fold or more (two missece mutations) (10).

In the present study, MIC's of ofloxacin, levofloxacin and norfloxacin were investigated by radiometric proporsion method in 20 MDR M.tuberculosis strains and in vitro activities were compared with each other.

MATERIALS AND METHODS

Total 20 MDR *M.tuberculosis* strains (eight resistant to streptomicin (S),isoniazid (I),rifampin (R) and ethambuthol (E), eight to IRE and four to SIR)were studied in this study and M.tuberculosis ATCC 27294 standart strain was also included. Ofloxacin (Koçak Pharmaceutical Co.,Istanbul), levofloxacin (Fako Pharmaceutical Co.,Istanbul) and norfloxacin (Merck Research Laboratories,Istanbul) were dissolved in 0.1 N NaOH. After preparing the stock solutions,they were kept in aliquots at -70°C. Working solutions ranging from 0.25 µg/ml-2 µg/ml for ofloxacin; 0.5 µg/ml-2 µg/ml for levofloxacin and 1.25 µg/ml-10 µg/ml for norfloxacin(1,11,12) were prepared with serial two fold dilutions using distilled water.All of the working solutions were freshly prepared for each run.For preparing the inoculum,the bacteria grown in Löwenstein-Jensen slants were suspended in 2-3 ml diluting fluid and homogenized with a glass mechanism.Turbidity was adjusted to a no 1 MacFarland standart with diluting fluid and 0.1 ml of standart inoculum was injected into a Bactec 12B vial (Becton Dickinson Diagnostic Instruments Systems,Sparks,MD). It was incubated at 37 °C and Growth Index (GI) was recorded daily.After the GI was reached 500,the contents of this vial were used as the primary inoculum. A total of 0.1 ml standardized inoculum was added to the vials along with 0.1 ml aliquots of different concentrations of ofloxacin, levofloxacin and norfloxacin. For each strain a drug free control (1:100 diluted inoculum) was also prepared. All of the vials were incubated at 37 °C and the GI was read and recorded daily on the Bactec TB 460 Instrument. Incubation continued for no more than 8 days or until the GI of the 1:100 diluted control was greater than 30.The lowest concentration of a drug with which the daily GI increase and final GI reading were lower than those of the 1:100 control was considered to have inhibited more than 99 % of the bacterial population and was designed as the MIC (13).

RESULTS

Minimal inhibitory concentrations of ofloxacin, levofloxacin and norfloxacin were 1 µg/ml, 0.5 µg/ml,and 5µg/ml for *M.tuberculosis* ATCC 27294 strain respectively. Minimal inhibitory concentrations obtained for ofloxacin were 1 µg/ml in 14 (70%) and 2 µg/ml in 6 (30%) strains;for levofloxacin 0.5 µg/ml in 8 (40%), ≤ 0.5 µg/ml in 7 (35%) and 1 µg/ml in the remaining 5 (25%) strains.For norfloxacin MIC values were determined as 5 µg/ml in 11 (55%) , 10 µg/ml in 7 (35%) and 2.5 µg/ml in 2 (10%) strains (Table 1). The MIC_{50} and MIC_{90} values of ofloxacin, levofloxacin and norfloxacin were found to be as 1µg/ml, 0.5µg/ml, 5µg/ml and 2µg/ml ,1µg/ml, 10µg/ml respectively

Table 1. The MIC's of ofloxacin, levofloxacin and norfloxacin to 20 clinical MDR- M.tuberculosis strains.

	Ofloxacin				Levofloxacin				Norfloxacin			
	0,25	0,5	1	2	≤ 0,5	0,5	1	2	1,25	2,5	5	10
ATCC 27294	0	0	1	0	0	1	0	0	0	0	1	0
Clinical strains	0	0	14	6	7	8	5	0	0	2	11	7

Table 2. The MIC_{50} and MIC_{90} values of the drugs tested to 20 clinical MDR-M.tuberculosis strains.

	Range (µg/ml)	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)
Ofloxacin	1-2	1	2
Levofloxacin	≤ 0.5-1	0.5	1
Norfloxacin	2.5-10	5	10

(Table 2). The MIC_{50} and MIC_{90} values of levofloxacin were one dilution less than that of ofloxacin, while the highest MIC values were obtained in norfloxacin against MDR *M.tuberculosis* strains.

DISCUSSION

The new fluoroquinolones are potent synthetic antibacterial agents with broad spectra of activity, including against mycobacteria (1). Among many of the fluoroquinolones tested against mycobacteria, both ofloxacin and levofloxacin showed the highest activities against M.tuberculosis (1,2,3,5,13,14),and were bactericidal against intracellulary growing tubecle bacilli (15).

Preliminar studies demonstrated that levofloxacin displayed a broad spectrum of bactericidal activities and is approximetely twice as active as ofloxacin(2,3). Altought there are so many studies evaluated the in vitro and in vivo activities of levofloxacin and ofloxacin,studies on the activities of norfloxacin are limited.

In a study performed by Mor et.al. (12), the MIC's of levofloxacin for *M.tuberculosis* determined radiometrically were twofold lower than those of ofloxacin (range,0.5 to 1µg/ml). Klemens et.al (2) evaluated the activity of levofloxacin in a murine model of tuberculosis and levofloxacin at 200 mg/kg had more than twofold greater activity than ofloxacin at the same dose. In tests with 18 drug – susceptible strains of *M.tuberculosis*, the MIC_{50} of levofloxacin was one dilution less than that of ofloxacin ,but the MIC_{90} was the same as that of ofloxacin (3).Richeldi et.al (5) carried out some in vitro tests with 20 (18 clinical, 2 library) *M.tuberculosis* strains before introducing levofloxacin into the treatment of tuberculosis.In Dubos broth medium,levofloxacin inhibited the growth of all the *M.tuberculosis* strains in concentrations of 0.5- 1 mcg/ml, but ofloxacin didn't inhibit any strain below the concentration of 1 mcg/ml. Pracharktam et.al (16), evaluated the MIC's of levofloxacin and ofloxacin against 47 MDR and 62 non - MDR *M.tuberculosis* trains. The MIC_{90} values of levofloxacin and ofloxacin were 1µg/ml and 2µg/ml respectively.Of these non-MDR strains, the MIC_{90} of both drugs were 0.5 µg/ml and 1µg/ml respectively.It seemed that MIC's of levofloxacin to MDR and non-MDR strains were one dilution less than ofloxacin. Ruiz-Serrano et.al (17) reported that levofloxacin showed the greatest activity (MIC_{90} 1µg/ml) with 96.4 % of the strains inhibited at 1µg/ml, while the MIC_{90} of ofloxacin was 2µg/ml (88.8 %) against 250 clinical isolates of *M.tuberculosis* strains. The activity of fluoroquinolones was higher in susceptible strains than in resistant resistant strains, with a twofold difference in the MIC_{90} of ofloxacin but there was no difference in the MIC_{90} of levofloxacin.

Effectiveness of ofloxacin,pefloxacin,norfloxacin and ciprofloxacin to 25 *M.tuberculosis* strains were examined using Middlebrook 7H10 agar. Of the isolates, 100 % were inhibited with 1,2,8,and 4µg/ml of ofloxacin, ciprofloxacin, pefloxacin and norfloxacin respectively and MIC_{50} values were found to be as 0.5, 0.25, 4 and 2µg/ml, for these quinolones with the same order (18). In an another study, MIC_{50} and MIC_{90} values of ofloxacin, levofloxacin and norfloxacin were reported as 0.5, 1, 4µg/ml and 0.5-1 , 1 , 8µg/ml respectively (1).

In the present study, the MIC_{50} and MIC_{90} values of ofloxacin,levofloxacin and norfloxacin were found to be as 1µg/ml, 0.5µg/ml, 5µg/ml and 2µg/ml, 1µg/ml, 10µg/ml respectively. The MIC's of levofloxacin were more lower than that of ofloxacin, while the highest MIC values were obtained in norfloxacin against MDR *M.tuberculosis* strains.

In conclusion,ofloxacin and levofloxacin can be used as alternative drugs in the treatment of MDR-tb caused by the strains isolated from our laboratory and levofloxacin seems to be a drug of first choise because of the more lower MIC values.

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