In Vitro Susceptibility Testing of Levofloxacin and Ofloxacin by Microtiter Plate Alamar Blue Against Multidrug and Non Multidrug Resistant *Mycobacterium tuberculosis* in Thailand†

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Abstract

Antituberculous drugs for current therapy of multidrugs resistant tuberculosis (MDR-TB) are limited. The *in vitro* susceptibility of *Mycobacterium tuberculosis* (MTB) as well as MDR-TB with other antibiotic drugs were determined in order to find alternative drugs for the treatment of MDR-TB. Forty-seven MDR-TB and 62 MTB of clinical isolates were tested against levofloxacin and ofloxacin. The MDR-TB at the MIC_{90} of levofloxacin and ofloxcain were 1 μg/ml and 2 μg/ml, respectively. Of these MTB, the MIC_{90} of both drugs were 0.5 μg/ml and 1 μg/ml, respectively. It seemed that levofloxacin MICs of both MDR-TB and MTB were one dilution less than ofloxcin. The promising activity of ofloxacin and levofloxacin against MDR-TB and MTB suggest that both drugs could be used as second-line drugs for MDR-TB or as a good alternative antituberculous drug for patients who have intolerance to the first line drug.

Key word : *Mycobacterium tuberculosis*, Tuberculosis, Levofloxacin, Ofloxacin, Alamar Blue

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Mycobacterium tuberculosis (*M. tuberculosis*) is one of the major causes of Mycobacterial infection in developing countries among acquired immunodifficiency syndrome (AIDS) patients. It is one of the serious public health problems and 3 million patients died every year from tuberculosis in the recent decade[1]. Furthermore, the rise of antituberculous drug resistance both for patients co-

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infected with HIV and for those without HIV infected tuberculosis have been reported among patients in the central region of Thailand[2]. The development of new drugs for the treatment of tuberculosis is scanty. Fluoroquinolone antibiotics have become the important "second-line" drugs for the treatment of resistant tuberculosis. Ofloxacin has been used in these patients and the resistance of treated patients has increased[2]. Levofloxacin is reported to be as potent as ofloxacin against various microorganisms including *M. tuberculosis*[3,4]. Information about the *in vitro* activity of levofloxacin against *M. tuberculosis* isolated from Thailand is limited. The purpose of this study was to determine the *in vitro* activity of levofloxacin in comparison with ofloxacin against *M. tuberculosis* and multidrug resistant *M. tuberculosis* (MDR-TB) isolated from Thai patients by microdilution methods using Alamar blue.

MATERIAL AND METHOD

1. Antimicrobial agents : Levofloxacin (LEV) and ofloxacin (OFX) were generously provided by Daiichi Pharmaceutical Co., Tokyo, Japan. Isoniazid (INH), rifampicin (RMP), ethambutal (EMB) and streptomycin (SM) were obtained from Sigma Chemical Co. St. Louis, Mo. LEV, INH, EMB and SM were initially dissolved in distilled water and made sterile by filtration as stock solutions. Stock solution of OFX was first dissolved in glacial acetic acid and then again with distilled water and made sterile by filtration. RMP was dissolved in Dimethyl sulfoxide and allowed to self-sterilize prior to further dilution with sterile distilled water as stock solution. These stock solutions were diluted further to make an appropriate working solution with culture broth media (7H9 with 10% OADC).

2. Bacterial strains and inoculum suspensions : Multidrug resistant *M. tuberculosis*, 16 strains, were kindly given by the division of Tuberculosis, Central Diseases Control of Public Health, 6 strains from the Central Chest Hospital, 22 strains from the Department of Microbiology, Siriraj Hospital, and 3 strains from Ramathibodi Hospital. Sixty-two clinical isolates non MDR-TB from pulmonary and extrapulmonary specimens of patients both non HIV infected and HIV infected patients at Ramathibodi Hospital and *M. tuberculosis* H_{37} Rv ATCC 27294 (American Type Culture Collection, Rockville, Md.) All the organisms were subcultured on Lowenstein Jensen Media. The inoculums were prepared by using fresh colonies of Mycobacteria grown on Loweinstein Jensen medium suspended in 2 mL of sterile distilled water with 0.04 per cent v/v of Tween 80 and homogenized with glass beads (4 mm in diameter). The suspension was allowed to stand for a few minutes to sediment the bacterial clump. The supernatant was adjusted for turbidity equal to that of a No 1 McFarland standard with sterile distilled water and further diluted 1:50 with culture broth media to a final concentration of 2 - 6 x 10^5 cfu/mL as an inoculum. The minimum inhibitory concentration (MIC) was performed by microtiter plates assay as described by Fraunzblace et al[5]. Using 96 clear wells flat bottomed sterile plates (Nunc), the activity of OFX and LEV was determined as pararell to standard antituberculous drugs (INH, RMP, EMP and SM). Growth was determined by the change of colour of Alamar blue from blue to pink. MIC was defined as the lowest drug concentration that remained blue.

RESULTS

The *in vitro* susceptibility of *M. tuberculosis* by broth dilution microtiterplate demonstrated that both 62 non MDR-TB and 47 MDR-TB were susceptible to ofloxacin and levofloxacin. (Table 1) MICs of both ofloxacin and levofloxacin for non MDR-TB ranged from 0.125-2 microgram per milliliter (μg/ml) for the MDR-TB, MICs for ofloxacin and levofloxacin ranged from 0.25-8 (μg/ml) and 0.125-8 μg/ml respectively. The MIC_{50} of ofloxacin and levofloxacin was 1 μg/ml and 0.5 μg/ml for both non MDR-TB and MDR-TB. The MIC_{90} of

Table 1. MICs of OFLO and LEV for *M. tuberculosis*.

Strains	No. Strains	MIC range (μg/ml)		MIC 50 (μg/ml)		MIC 90 (μg/ml)	
		OFLO	LEV	OFLO	LEV	OFLO	LEV
Non MDR TB	62	0.125-2	0.125-2	1	0.5	1	0.5
MDR TB	47	0.125-8	0.125-8	1	0.5	2	1

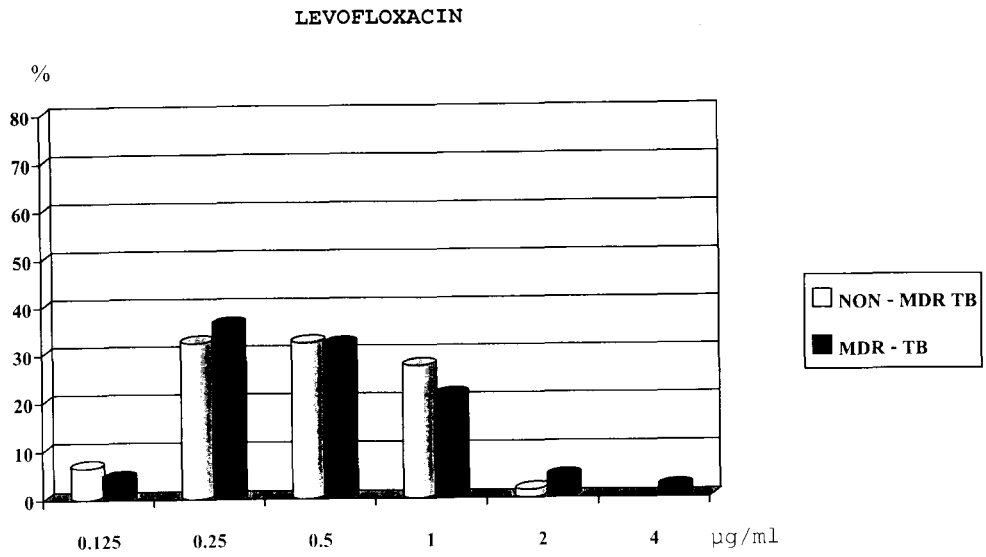

Fig. 1. Levofloxacin MICs between M. TB and MDR-TB

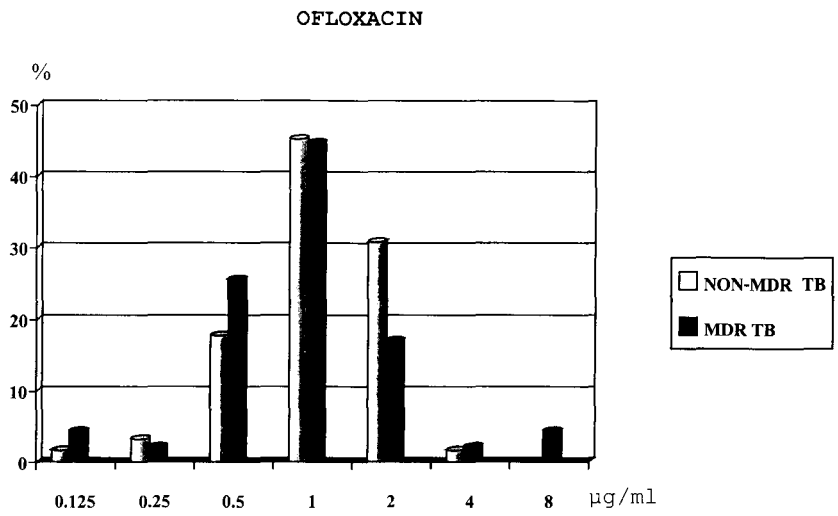

Fig. 2. Ofloxacin MICs between MTB and MDR-TB.

ofloxacin and levofloxacin was 2 µg/ml and 1 µg/ml. It seemed that ofloxacin MICs of both MDR-TB and non MDR-TB were one dilution greater than levofloxacin. The MICs of each drug both non MDR-TB and MDR-TB are shown in Fig. 1 and Fig. 2. The distribution of MIC both non MDR-TB and MDR-TB was almost the same except that two MDR-TB (0.91%) had ofloxacin MIC of 8 µg/ml and one of these also had levofloxacin MIC of 4 µg/ml.

DISCUSSION

The incidence of primary and acquired INH & RMP resistance has been reported in Thailand as well as the poor tolerance of rifampicin therapy(6). The need for other antituberculous drugs is urgent.

Fluoroquinolone is known as one of the antibacterial drugs that has activity against *M. tuberculosis* (7). These data show that most *M. tuberculosis*, both non MDR-TB and MDR-TB, are susceptible to ofloxacin and levofloxacin except two strains which were resistant to ofloxacin and only one of them was also resistant to levofloxaxin. These 2 strains, when traced back to the first line drug susceptibility, were resistant to all four first line drugs with the MIC at high concentration. Most of the MICs of levofloxacin for *M. tuberculosis* in this study had activity of one dilution less than ofloxacin which is similar to previous reports(8,9). Only less than one per cent showed levofloxacin resistance, which seems to be a low incidence in this study. This low incidence may occur as ofloxaxin and levofloxacin are not widely used in Thailand since they are new fluoroquinolone drugs. Not many *M. tuberculosis* patients receive these drugs for treatment of other kinds of infection. The acquired resistance of these two drugs among tuberculosis patients seldom occurs. The results of this study showed that levofloxacin was active against *M. tuberculosis* about two fold greater in inhibitory and bactericidal activities than ofloxacin. However, these fluoroquinolones, although highly active against MDR and non MDR *M. tuberculosis*, should be reserved as second-line drugs for MDR-TB or for patients who cannot tolerate the first-line drugs.

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REFERENCES

1. Grange JA, Zumia A. Paradox of the global emergency of tuberculosis. Lancet 1999; 353: 996.
2. Riantawan P, Punnotok J, Chaisuksuwan R, Pransujarit V. Resistance of *Mycobacterium tuberculosis* to antituberculosis drugs in the central region of Thailand. Int J Tuberc Lung Dis 1998; 2: 616-20.
3. Fu KP, Lafredo SC, Foleno B, et al. *In vitro* and *in vivo* antibacterial activities of levofloxacin (l-ofloxacin) an optically active ofloxacin. Antimicrob Agent Chemother 1992; 36: 860-6.
4. Mor N, Vanderkolk J, Heifets L. Inhibitory and bactericidal activities of levofloxacin against *Mycobacterium tuberculosis in vitro* and in human macrophages. Antimicrob Agent Chemother 1994; 38: 1161-4.
5. Franzblau SG, Witzig RS, Mc Laughlin JC, Torres P, Modico G, Gilman RH. Rapid, low technology, MIC determination and with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar blue assay. J Clin Microbiol 1998; 32: 362-6.
6. Leechwengawong M, Punyagupta S. Pneumococcal Vaccination in Thailand: A Reappraisal of Adopting US Recommendations for Thai Health Problems. J Infect Dis Antimicrob Agent 1999; 16: 97-9.
7. Plouff JF and Franklin Country Pneumonia Study Group. Levofloxacin *in vitro* activity against bacteremic isolates of *Streptococcus pneumoniae*. Diagn Microbiol Infect Dis 1997; 25: 43-5.
8. Baohong JI, Lounis N, Truffot-pernot C, Grosset J. *In vitro* and *in vivo* activities of levofloxacin against *Mycobacterium tuberculosis*. Antimicrob Agent Chemother 1995; 39: 1341-4.
9. Mor N, Vanderkolk J, Heifets L. Inhibitory and bactericidal activities of Levofloxacin against *Mycobacterium tuberculosis in vitro* and in human macrophages. Antimicrob Agent Chemother 1994; 38: 1161-4.

การทดสอบความไวของยาเลโวฟล็อคซาซินและออฟล็อคซาซินโดยวิธีไมโครไดลูชั่นและอลามาร์บลูต่อเชื้อวัณโรคและเชื้อวัณโรคดื้อยาในประเทศไทย

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ในปัจจุบันมีรายงานพบเชื้อวัณโรคดื้อยาหลายชนิดพร้อมกัน (multidrugs resistant tuberculosis หรือ MDR-TB) ซึ่งยาที่มีคุณภาพที่ดีในการรักษาผู้ป่วยกลุ่มนี้ยังมีจำนวนน้อย ดังนั้นการทดสอบความไวของเชื้อวัณโรค (MTB) และ MDR-TB กับยาปฏิชีวนะในกลุ่ม fluoroquinolones, ofloxacin และ levofloxacin จึงเป็นหนทางหนึ่งในการค้นหายาเพื่อใช้รักษาผู้ป่วย MDR-TB เชื้อ MTB 62 สายพันธุ์ และ MDR-TB 47 สายพันธุ์ ได้ทดสอบความไวเชื้อต่อยา ofloxacin และ levofloxacin MIC90 ของ MDR-TB ต่อยา levofloxacin และ ofloxacin อยู่ที่ 1 μg/ml และ 2 μg/ml ส่วน MIC90 ของ MTB ต่อยาทั้ง 2 ชนิดเป็น 0.5 μg/ml และ 1 μg/ml ซึ่งดูเหมือนว่า MIC ต่อ levofloxacin จะมีความเข้มข้นของยาน้อยกว่า ofloxacin อยู่หนึ่งเท่า จากผลการทดสอบความไวของเชื้อทั้ง MDR-TB และ MTB ต่อยาทั้งสองชนิดนี้สามารถยับยั้งการเจริญเติบโตของเชื้อวัณโรคได้ และสามารถใช้เป็นยาสำรองสำหรับรักษา MDR-TB และเป็นยาเลือกในกรณีผู้ป่วยวัณโรคทนต่อผลข้างเคียงของยาที่เป็นมาตรฐานในการรักษาไม่ได้

คำสำคัญ : *มัยโคแบคทีเรียม ทูเบอร์คูโลสิส*, วัณโรค, เลโวฟล็อคซาซิน, ออฟล็อคซาซิน, อลามาร์ บลู

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