Full Length Research Paper

***In vitro* susceptibility of *Mycobacterium tuberculosis* to clarithromycin**

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This study was done to evaluate *in vitro* susceptibility of *Mycobacterium tuberculosis* of clarithromycin. In this prospective study, 90 *M. tuberculosis* strains (40 resistant strains and 50 susceptible strains) referred to the Tabriz laboratory of TB research Center were selected and cultured in L-J medium and with 3 doses of clarithromycin. 40 strains were resistant and 50 strains were sensitive to first line drugs. 60% of strains were resistant to clarithromycin with 20 µg/ml. Significant difference was not found if resistant rate of *M. tuberculosis* strains to clarithromycin with 20 and 40 µg/ml and all studied strain was sensitive to clarithromycin with 80 µg/ml. 33 strains of resistant strains and 48 strains of sensitive strains to first anti tuberculosis line drug was sensitive to clarithromycin with 40 µg/ml. Based on the results of this study and other studies, the need for extensive laboratory study and using liquid culture (including Middelbary) and advanced techniques such as BACTEC and even more animal models are necessary. The long-term clinical study using clarithromycin in combination with anti-TB therapy, especially in the case of MDR-TB strains is necessary for the low side effect as clarithromycin in the treatment of tuberculosis, in the form of suitable compounds which can be used as second drug.

Key words: *Mycobacterium tuberculosis*, drug resistant, clarithromycin, strains.

INTRODUCTION

Re-incidence of infection and disease caused by *Mycobacterium tuberculosis* and the increasing prevalence of multi-drug resistance among strains of *M. tuberculosis* evokes the idea that new drugs are still needed (Brennan and Nikaido, 1995). Among the candidates for second-line treatment of Tuberculosis of the macrolides, clarithromycin can be named. While mycobacterial cell membrane acts as a barrier impermeable to the medication, they penetrate the mycobacterial cell membrane and influence them (Bermudez et al., 1998).

Clinical experience with macrolides antibiotics for *M. tuberculosis* is insufficient, thus the necessity of applying combinations of several drugs in the regimen therapy doubles the need for prior assessments of individual impact of Clarithromycin. Whether clarithromycin is effective

on patients or not was examined by measuring its effect on the improvement of colony forming units (CFU) of *M. tuberculosis* from sputum of patients with smear-positive pulmonary Tuberculosis (TB) (Mitnick et al., 2008).

Increasing incidence of resistant tuberculosis and increasing resistance to anti-mycobacterial drugs, especially in patients infected with human immunodeficiency virus (HIV), points out the immediate need for new compounds to overcome the resistance. Reassessment of old compounds such as macrolides, has been widely spread, which can be a way to deal with this problem (Sato and Tomioka, 1999a; Brudney and Dobkin, 1992). Systematic study of macrolides and matching it with data on the susceptibility of *M. tuberculosis* to antibiotics in this group could be noteworthy in the study on effective compounds (Bosne-David et al., 2000).

These results are consistent with other genetic and phenotypic studies showing the least heterogeneity in *M. tuberculosis* (Bermudez et al., 1998). The aim of this study was to evaluate drug susceptibility of *M. tuberculosis* to clarithromycin in *in vitro* condition.

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MATERIALS AND METHODS

This study was prospectively conducted on 90 isolated strains of *M. tuberculosis* from patients at TB Research center of Tabriz, 50 of which were susceptible to the drug of first-line treatment of tuberculosis and 40 of which were resistance to the drug of first-line treatment of tuberculosis. These strains were randomly selected from among patients referred to the laboratory of TB Research Center of Tabriz. Resistant strains were selected from among hundreds of strains isolated during recent years in the Laboratory of TB Research Center.

Antibiogram was conducted with proportional method in Lowenstein-Jensen environment. Three doses of clarithromycin were used to determine the susceptibility and resistance. For internal quality control in laboratory, for some strains, the Middlebrook 7H9 agar environment and disk diffusion method were also used. For this purpose, strains susceptible and resistant to drugs of first-line treatment of tuberculosis were cultured in the Lowenstein-Jensen medium; then 3 different doses of clarithromycin were used to determine the susceptibility and measure the minimum inhibitory concentrations (MIC). Drug resistance was considered as bacterial growth over 1% in medium containing antibiotic compared to the drug-free medium.

In this study, susceptibility tests and MIC determination have been conducted by agar dilution on 7H10 agar enriched with the oleic acid, albumin, dextrose and catalase. The *Mycobacterium* strains were grown in 7H9 broth at 37°C and diluted with distilled water for compliance with the Mc-Farland standard turbidity. The results were read after 3 weeks of incubation at a pressure of 1 atmosphere of CO₂ at 37°C; MIC is defined as the minimum concentration of antibiotic completely inhibiting visible bacterial growth (a colony was not considered). Antibiotics under study, each of which prepared by manufacturers as standard powders, the stability of antibiotics during the incubation period with the Petri was monitored by *E. coli* control species at days 1 and 11.

The samples were then evaluated by experts in TB Research Center of Tabriz; due to contamination in some samples, the tests were repeated and the final results, after tests accuracy confirmation, were determined as the susceptibility and resistance to different doses, and the MIC were then calculated for each of the resistant strains; then, the obtained data and drug resistance history of each strain was determined based on archive of research center, and supplementary information, including gender of the patients. The obtained results were then analyzed by SPSS 11.5 software and Chi-square statistical test, and the significance level was considered as $P < 0.05$.

RESULTS

Susceptibility and resistance of *M. tuberculosis* strains (MTS) to several dosage of clarithromycin between two groups of strains have been shown in Table 1. There was no significant difference in the rates of resistance and susceptibility to clarithromycin with doses of 20 µg/ml ($P = 0.083$) and 40 µg/ml ($P = 0.072$) between strain groups (strains resistant to first-line anti-TB drugs and strains susceptible to first-line anti-TB drugs) and all strains under study were susceptible to 80 µg/ml dose of clarithromycin (Table 1). Susceptibility and resistance of *M. tuberculosis* strains (MTS) to first-line anti-TB drugs have been shown in Table 2. There was no significant difference in susceptibility or resistance to clarithromycin among strains with different number of resistance to the

first generation of drugs ($P = 0.052$). Susceptibility and resistance of *M. tuberculosis* strains (MTS) to first-line anti-TB drugs and several doses of clarithromycin have been shown in Table 3.

DISCUSSION

M. tuberculosis remains a leading cause of mortalities associated with infectious disease agents in the world. In 1997, about 2 billion people were estimated to be infected with tuberculosis (Brennan and Nikaido, 1995). Every year, 8 to 9 million people get infected with TB and finally, 2 million people die of TB or symptoms of infection (Brennan and Nikaido, 1995). TB is a major problem in developing countries so that 95% of patients and 90% of mortalities due to this disease occur in these countries. In African regions, where the prevalence of infection with HIV is more than the most areas of the world, TB mortality rate is over 50% (Brennan and Nikaido, 1995).

In 1993, World Health Organization (WHO) called tuberculosis a global public health emergency problem. Since then on, TB and related mortality have remained constant. Despite the relative success in this case, we have been witnessing an increasing drug resistance; so that this phenomenon is now considered a global threat. Resistance to an anti-tuberculosis drug has been recognized many years ago. Unfortunately, the evolution of drug resistance in TB treatment has led to strains resistant to multiple drugs (MDR-TB: Multi Drug Resistant-TB).

The incidence of MDR-TB is increasing rapidly and the estimated incidence in the whole world was 460,000 cases in 2005 (Bermudez et al., 1998). Due to lack of appropriate diagnostic and laboratory facilities and sometimes lack of adequate and reliable reporting system, the prevalence of MDR-TB in many developing countries is estimated lower than the actual rate. Almost two third of MDR-TB cases are seen in the three countries; Russia, China and India.

In a study in Iran, sputum samples were taken from 2,030 patients with known TB and examined for *M. bacilli* (AFB) by microscopic examination and were cultured in Lowenstein-Jensen standard medium. *M. tuberculosis* was susceptible to first-line drugs in 1,284 and then MDR-TB was observed in 113 patients. Totally, 12 (about 10%) of MDR-TB cases were resistant to all second-line TB treatment drugs which are called XDR-TB (Extensively Drug Resistant TB). In the recent study, two groups of XDR-TB are described (Sato and Tomioka, 1999b). If these over-resistant XDR-TB pathogens find a chance of growth and reproduction, we will encounter a serious global health and economic problem.

Insignificant available experience and the results obtained from previous studies indicate that antibiotics of Macrolides family are effective against *M. tuberculosis* in patients with TB disease, from among which,

Table 1. Susceptible and resistant *Mycobacterium tuberculosis* strains (MTS) to several dosage of clarithromycin between two groups of strains.

Parameter ($\mu\text{g/ml}$)		Group		P
		Resistant	Susceptible	
Clarithromycin 20	Resistant	20	34	0.083
	Susceptible	20	16	
Clarithromycin 40	Resistant	7	2	0.072
	Susceptible	33	48	
Clarithromycin 80	Susceptible	40	50	-

Table 2. Susceptible and resistant *Mycobacterium tuberculosis* strains (MTS) to first-line anti-TB drugs.

Parameter	Susceptible and resistant of <i>Mycobacterium tuberculosis</i> strains	
	Resistant	Susceptible
Isoniazid	20	70
Rifampin	9	81
Streptomycin	28	62
Ethambutol	5	85

Table 3. Susceptible and resistant *Mycobacterium tuberculosis* strains (MTS) to first-line anti-TB drugs and several doses of clarithromycin.

Parameter		Clarithromycin 20 (µg/ml)		P	Clarithromycin 40 (µg/ml)		P	Clarithromycin 80 (µg/ml)		P
		Resistant	Susceptible		Resistant	Susceptible		Susceptible		
Isoniazid	Resistant	5	15	<0.001	2	18	0.682	20	-	
	Susceptible	49	21		7	63		70		
Rifampin	Resistant	3	6	0.148	3	6	0.043	9	-	
	Susceptible	51	30		6	75		81		
streptomycin	Resistant	14	14	0.247	3	25	0.574	28	-	
	Susceptible	40	22		6	56		62		
Ethambutol	Resistant	3	2	0.669	1	4	0.417	5	-	
	Susceptible	51	34		8	77		85		

clarithromycin was more favored and tested due to three reasons:

1. Biochemical drug and susceptibility predicted the activity rate;
2. Clinical findings, although incomprehensive, indicated the effectiveness of this drug and;
3. This drug can be administered orally and its bactericidal effect is fast.

For the first time, these drugs were evaluated by

Mitchison while categorizing effectiveness of anti-TB drugs. After making sure of the relationship between the effectiveness of drugs in both *in vitro* and *in vivo* (inside the body) conditions proved in a drug class, it can be extended to other substances showing a similar activity in the laboratory (Brennan and Nikaido, 1995).

In a study by Parsons et al. (1997) at infectious diseases section of the Wadsworth Center in New York, they stated that drug resistance to anti-tuberculosis drugs is a global problem and affirmed that new drugs effective on *M. tuberculosis* are required. In a study by Mor and

Esfandiari (1997) at the University of California, United States, examining the combined effect of clarithromycin and pyrazinamide on *M. tuberculosis*, they stated that this combination has a synergistic effect on *M. tuberculosis*. In a study by Cavalieri et al. (1995) at the University of Omaha, United States, they stated that clarithromycin in combination with other anti-TB drugs has good effect on strains of *M. tuberculosis*. In a study by Sato and Tomioka (1999a) at the Department of Microbiology, University of Izumo, Japan, examining the effect of clarithromycin on *M. tuberculosis* strains, they stated that clarithromycin is effective on *M. tuberculosis* strains and *M. tuberculosis* is susceptible to clarithromycin (Sato and Tomioka, 1999a).

In a study by Tomioka et al. (2000) at the Department of Microbiology, University of Izumo, Japan, they stated that clarithromycin was effective on *M. tuberculosis* strains and *M. tuberculosis* is susceptible to this antibiotic (Tomioka et al., 2000). In our study, *M. tuberculosis* strains were susceptible to clarithromycin with doses of 20, 40 and 80 µg/ml at 40, 90 and 100% of cases. In a study by Bhusal et al. (2005) at the University of Ryukyus in Okinawa, Japan, with examining the effect of the clarithromycin on *M. tuberculosis*, they expressed that the antibiotic under their study has synergistic effect on this micro-organisms along with other anti-TB antibiotics.

Macrolides are one of the most important antibiotics for human use. This group of drugs is widely used in medicine due to their wide range of activity and few side effects. Experimental studies have shown that macrolides have anti-mycobacterial activity and their combinations with Ethambutol (one of the first-line anti-TB drugs) may increase its effectiveness on Tuberculosis bacilli (Brennan and Nikaido, 1995). The results suggest that both drugs should be investigated further as potential adjuncts to the treatment of resistant tuberculosis, in particular through new drug delivery systems such as the dry powder inhaler allowing high lung deposition (Umubyeyi et al., 2007; Stoffels et al., 2009).

For clofazimine, amikacin, clarithromycin, ciprofloxacin, and amoxicillin/clavulanate, all the MIC (90) of the MDR isolates exceeded those of non-MDR isolates (Lu et al., 2008). Cremades et al., (2008) demonstrated that addition of clarithromycin to anti-tuberculosis regimens in MDR was beneficial.

In addition, the clinical findings suggest that beta-lactamase have good future for use in treating patients with MDR-TB. However, more studies are needed to determine the interaction of these drugs with first line anti-TB drugs.

Conclusion

According to the study on the effects of clarithromycin on strains of *M. tuberculosis* susceptible and resistant to first line anti-TB drugs, in both Lowenstein-Jensen environment

and *in vitro*, respectively, 60 and 10% of strains of *M. tuberculosis* were resistant to clarithromycin with doses of 20 and 40 µg/ml. There was no significant difference in the resistance of *M. tuberculosis* to clarithromycin with doses of 20 and 40 µg/ml; while all strains of *M. tuberculosis* (susceptible and resistant to first-line anti-TB drugs) were susceptible to clarithromycin with dose of 80 µg/ml. There was no significant difference between susceptible and resistant strains, and even the noninterference of the number of resistance to the first-line anti-tuberculosis drugs in the susceptibility to different doses of clarithromycin show that clarithromycin might be a good option in combined treatment of resistant TB cases. We have tested three different concentrations of clarithromycin to set a break point for clarithromycin, a greater number of different concentrations of clarithromycin are recommended.

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