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A Study of the Effectiveness of Two Common Fluoroquinolones on Mycobacterial Strains Isolated From Patients

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Abstract

Background and objectives: Investigation and study of second-line drugs effects on mycobacterial strains has been of great importance due to prevalence of drug resistance especially multi-drug resistance (MDR) in *Mycobacterium tuberculosis* (MTB) strains in the recent years. The objective of this research was to determine drug sensitivity of *Mycobacterium tuberculosis* and mycobacteria other than tubercle bacilli (MOTT) strains to the two common second-line anti-mycobacterial fluoroquinolones, i.e. ofloxacin (OFL) and ciprofloxacin (CIP).

Materials and methods: In this study, the *in-vitro* activities of two drugs of OFL and CIP, considering the effects of first-line drugs (isoniazid, rifampin, streptomycin and ethambutol) were studied on 100 mycobacterial strains containing 90 MTB and 10 MOTT strains isolated from patients admitted to research center for TB and pulmonary diseases of Tabriz, Iran, by proportion method of drug susceptibility on Lowenstein-Jensen (LJ) medium.

Results and conclusion: Out of 90 MTB strains, 50 strains that were sensitive to the first line drugs were diagnosed as susceptible to OFL and CIP. Of other 40 strains which were resistant to the first line drugs, only one strain was resistant to OFL and 2 strains were found to be resistant to CIP. Of 10 MOTT strains, 4 strains were resistant to OFL and 3 strains were found to be resistant to CIP. The findings of this investigation revealed that OFL and CIP could be effectively used against MTB and MOTT.

Keywords: MTB; MOTT; Drug resistance; Fluoroquinolones

Introduction

After discovery of tubercle bacillus in 1882 by Robert Koch, humankind hoped to control and root out tuberculosis infection and disease. But nowadays, after more than 50 years of the first anti tuberculosis drug discovery and using different advanced diagnostic methods and access to new anti tuberculosis drugs, tuberculosis is still a serious problem and main anxiety for human health. At present time, tuberculosis is one of the most important human mortality factors and causes the death of people more than other infectious diseases. About one third of the world population is infected with *Mycobacterium tuberculosis* (MTB) and each year 8-10 million new cases of tuberculosis are recognized. Also annually 2-3 million people are dying because of this disease. Now tuberculosis control and inhibition programs have been faced with two serious threats; one is epidemicity of human immune deficiency virus or AIDS disease and the other is prevalence of drug resistance especially multi-drug resistant tuberculosis (MDR-TB) [1-3].

MDR-TB is a special form of tuberculosis in which isolated strain is resistant to at least two main and important anti tuberculosis drugs, i.e. isoniazid and rifampin [1-3]. Increasing resistance of *Mycobacterium tuberculosis* (MTB) and mycobacteria other than tubercle bacilli (MOTT) strains to main anti tuberculosis drugs has caused fluoroquinolones and aminoglycosides as second-line drugs of tuberculosis to become suggested therapies for the resistant tuberculosis treatment. In 2000, the World Health Organization (WHO) reported that more than 50 million people have been infected with MDR-TB in the world. MDR-TB is considered as a main threat and problem in controlling and inhibiting tuberculosis in the world. Also WHO declared the high rate of MDR-TB in Iran in an investigation done on the prevalence of MDR-TB in some countries during 1996-1999. Treatment of the MDR-TB is a necessity among which, is secured therapy of patients suffering from MDR-TB. This research was performed in order to investigate on the effects of two common fluoroquinolonic drugs of ofloxacin (OFL) and ciprofloxacin (CIP) (as second-line anti tuberculosis drugs) against first-line drug (isoniazid, rifampin, streptomycin and ethambutol) resistant and sensitive MTB and MOTT strains isolated from patients admitted to research center for TB and pulmonary diseases of Tabriz, Iran [4-7].

Since drug resistant tuberculosis is a worldwide problem of fighting against tuberculosis disease and conventional treatment (anti tuberculosis drugs) could not help for the therapy of tuberculosis patients infected with drug resistant strains especially in MDR-TB type of tuberculosis, so for the treatment of such patients, second-line drugs, such as fluoroquinolones have been recommended by the competent authorities like WHO and International Union Against Tuberculosis and Lung Disease (IUATLD) [7].

Fluoroquinolones have outstanding place in the treatment of bacterial infections. These drugs are well absorbed through gastrointestinal tract and are spread in tissues especially lung tissue and their concentration in respiratory secretions are so higher than serum level. These drugs could diffuse into the macrophages and there could be concentrated. Also host tolerance in long time treatments has been reported as good with the fluoroquinolones. WHO categorizes fluoroquinolones as bactericide drugs and recommends their usage in tuberculosis patients in which relapse has been occurred because

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of therapy failure. Most of the fluoroquinolones compounds such as OFL and CIP are effective on mycobacteria. Fluoroquinolones cause inhibition of topoisomerase II or DNA gyrase and topoisomerase IV of bacterium. Topoisomerases are very important and necessary enzymes for copying, proliferation, restoring of bacterium DNA, and keeping bacterium DNA in super coiling state [7-11].

Materials And Methods

Ninety MTB strains (50 strains sensitive and 40 strains resistant to the first-line drugs) and 10 MOTT strains (all strains resistant to the first-line drugs), totally 100 strains, were selected for conducting research in a irregular random form among hundreds of isolated strains in a four-year time period from patients admitted to research center for TB and pulmonary diseases of Tabriz, Iran. The reason for lower number of MOTT strains was less isolation of these species. Strains were isolated from sputum, bronchial fluid, cerebrospinal fluid, abscesses, urine and gastric lavage samples related to 47 male and 53 female cases. Storage of samples was done in -20°C after collecting for the study. Concentration of used drugs in Lowenstein-Jensen media were 0.2 µg/ml for isoniazid, 40 µg/ml for rifampin, 2 µg/ml for ethambutol, 4 µg/ml for streptomycin, 4 µg/ml for ofloxacin, and 4 µg/ml for ciprofloxacin. Determination method for drug susceptibility in this study was the standard reference method, i.e. proportion method on Lowenstein-Jensen medium which has been approved by WHO. The solvent for used antibiotics was sterile distilled water and drugs in mentioned dilutions were added to media [5,7,12,13]. In this study, standard *Mycobacterium tuberculosis* strain of H37RV sensitive to all drugs was used as quality control strain in each series of experiments [7].

Results

Regarding type of studied strains, the results of drug sensitivity experiments were registered in three stages. Table 1 is related to the first stage in which the results of drug sensitivity tests for 50 *Mycobacterium tuberculosis* (MTB) strains sensitive to all first-line drugs (isoniazid, rifampin, streptomycin and ethambutol) are shown. According to Table 1, all MTB strains sensitive to first-line drugs were also sensitive to ofloxacin (OFL) and ciprofloxacin (CIP).

Strain	No. of studied strains (total)	No. of strains sensitive to all first-line drugs	No. of strains sensitive to CIP	No. of strains sensitive to OFL
MTB	50	50	50 (100%)	50 (100%)

MTB: *Mycobacterium tuberculosis*, CIP: Ciprofloxacin, OFL: Ofloxacin

Table1: Drug sensitivity rate of 50 MTB strains sensitive to all first-line drugs against CIP and OFL.

Strain	No. of studied strains	Type of drug resistance to first-line drugs	Name of resistant drugs	No. of strains resistant to CIP	No. of strains resistant to OFL
MTB	6	SDR	H	0	0
	3	SDR	R	0	0
	17	SDR	S	1	0
	2	MDR	H,R,E,S	0	0
	1	MDR	H,R,E	1	1
	3	MDR	H,R,S	0	0
	2	MDR	H,R	0	0
		Other MDR	Except H,R	0	0
	Total 40			Total 2 (5%)	Total 1 (2.5%)

MTB: *Mycobacterium tuberculosis*, SDR: Single Drug Resistant, MDR: Multi-Drug Resistant, H: Isoniazid, R: Rifampin, S: Streptomycin, E: Ethambutol, CIP: Ciprofloxacin, OFL: Ofloxacin

Table 2: Drug resistance rate of 40 MTB strains resistant to first-line drugs against CIP and OFL.

Strain	No. of studied strains	Type of drug resistance to first-line drugs	Name of resistant drugs	No. of strains resistant to CIP	No. of strains resistant to OFL
MOTT	9	MDR	H,R,E,S	3	4
	1	MDR	H,R,E	0	0
	Total 10			Total 3 (30%)	Total 4 (40%)

MOTT: Mycobacteria other than tubercle bacilli, MDR: Multi-Drug Resistant, H: Isoniazid, R: Rifampin, E: Ethambutol, S: Streptomycin, CIP: Ciprofloxacin, OFL: Ofloxacin

Table 3: Drug resistance rate of 10 MOTT strains resistant to first-line drugs against CIP and OFL.

Table 2 in the second stage, shows the results of drug sensitivity tests of 40 MTB strains resistant to first-line drugs (resistant to at least one of the first-line drugs) against OFL and CIP. According to this table, totally of 40 MTB strains resistant to first-line drugs, only one strain (2.5%) is resistant to OFL and 2 strains (5%) are resistant to CIP. Also the Table 2 show that among 17 single drug resistant (SDR) (to streptomycin) strains, only one strain was resistant to CIP. Also one multi drug resistant (MDR) (to isoniazid, rifampin and ethambutol) strain was resistant to both studied drugs of OFL and CIP.

Table 3 in the third stage shows the results of drug sensitivity tests of 10 mycobacteria other than tubercle bacilli (MOTT) strains resistant to first-line drugs against OFL and CIP. According to Table 3, of totally 10 MOTT strains resistant to first-line drugs, 4 strains (40%) were found to be resistant to OFL and 3 strains (30%) were recognized as resistant to CIP and this rate of resistance was observed only in quadruple drug resistance state (isoniazid, rifampin, ethambutol and streptomycin).

Discussion

Resistance to anti tuberculosis drugs has been observed since starting of chemotherapy against tuberculosis. Drug resistance was considered as a global problem since prevalence of multi drug resistant tuberculosis (MDR-TB) including isoniazid, rifampin, streptomycin and ethambutol (at least isoniazid and rifampin) in the early 1990s and nowadays prevalence of drug resistant tuberculosis in independent countries of the former Soviet Union, India, China, and countries of south of African desert (which are extremely infected by AIDS) is the matter of anxiety. Also WHO has estimated that 50 million people of the world population are infected by drug resistant *Mycobacterium tuberculosis* strains. The emergence of MDR-TB has become a serious and major problem in successful treatment and eradication of tuberculosis worldwide [3-5,14,15].

Recent studies show that MDR-TB in neighbor countries of Iran such as Azerbaijan Republic, Turkey, and other independent countries of the former Soviet Union is increasing. In an investigation that Portaels *et al.* conducted in Azerbaijan and Russia, they reported that MDR-TB rate in Marynsk region of Siberia, Russia and Baku city of Azerbaijan as 23.6% and 23.7% respectively [16].

According to reports that show increase of drug resistance, serious discussions are appeared about researches and using second-line drugs for therapy of infections resulted by *Mycobacterium tuberculosis* (MTB) and mycobacteria other than tubercle bacilli (MOTT). Of these drugs we can name ofloxacin (OFL) and ciprofloxacin (CIP) which are of

fluoroquinolones. Totally these antibiotics act against DNA synthesis by preventing activity of DNA-gyrase, which has been explained completely in introduction part [7-11].

Findings of this study showed that OFL and CIP could be effective against MTB strains of sensitive and resistant to first-line drug (isoniazid, rifampin, streptomycin and ethambutol) (Tables 1, Table 2). According to Table 2 it could be seen that totally, sensitivity rate of MTB.

Strains (resistant to first-line drugs) are 97.5% to OFL and 95% to CIP that could be considered as valuable sensitivities. A 2.5% resistance rate of strains to OFL in comparison with a 5% resistance rate of strains to CIP is justifiable with higher utilization of CIP in the society than OFL. Paying attention to Table 2, one could realize that from 17 MTB strains only resistant to streptomycin, just one strain is resistant against CIP. This matter could be justified by this reason that since streptomycin as first-line drug has higher utilization, so most of resistances are related to streptomycin and consequently among 17 strains resistant to first-line drugs which consist of majority, one case is resistant to CIP, which can be logical.

Results of performed studies all along the world are compatible with results of our research and show that OFL and CIP are effective on drug sensitive and drug resistant MTB strains. Some of these studies are relevant to study of Yu Mc *et al.* on effect of OFL against MTB strains isolated from Taiwanese patients [17], research of Geerligs *et al.* on effects of OFL and CIP against MTB strains isolated from patients suffering from MDR-TB in Holland [18], study of Prammananan *et al.* on usefulness of OFL and CIP on MDR-TB strains isolated from Thai patients [19], study of Jain et al. on effectiveness of OFL and CIP against MTB strains isolated from Indian patients [9] and also research of Casal *et al.* on the effectiveness of OFL against MTB strains sensitive and resistant to first-line drugs, isolated from patients of 14 different regions of Spain [20].

In relation with drug resistance rate of MOTT strains, obtained results showed that totally 40% of first-line drug resistant MOTT strains were resistant to OFL and 30% of the mentioned strains were resistant to CIP (Table 3). The interesting point of this part (Table 3) was that this rate of fluoroquinolones resistance only was seen in quadruple drug resistance state (isoniazid, rifampin, streptomycin and ethambutol) and when streptomycin was one of the four drugs. Again we can interpret this point in this way that because of higher utilization of streptomycin as the main first-line drug and for causing resistance in bacterium, wherever there is streptomycin, bacterial drug resistance will be more there and maybe some unobvious and complex relations exist between these two drugs and other drugs and it seems that more precise investigations are needed to be done on this matter. Another interpretation of these findings (Table 3) which could be interesting is that in intensification mode of MOTT strains resistance, i.e. quadruple drug resistance state, mentioned strains shows relative resistance to OFL and CIP (40% and 30% respectively). While in triple drug resistance state (without streptomycin) this resistance rate to both drugs could be zero (Table 3). Anyhow if we consider all MOTT strains multi drug resistances as quadruple, still a 40% drug resistance or a 60% drug sensitivity of MOTT to OFL and also a 30% drug resistance or a 70% drug sensitivity of MOTT to CIP could be acceptable sensitivities for these strains. There was no significant difference between "MOTT resistance to OFL" and "MOTT resistance to CIP" ($p=0.63$) (Table 3) indicating that these two drugs of OFL and CIP have same value against MOTT strains.

Conclusion

1. Ofloxacin (OFL) and ciprofloxacin (CIP) could be used surely as two effective drugs against *Mycobacterium tuberculosis* (MTB) strains sensitive and resistant to first-line drugs.

2. Drug resistance of first-line drug resistant MTB strains to CIP is slightly higher than resistance to OFL which could be related to higher utilization of CIP in society.

3. In relation with MTB strains, the highest drug resistance (among first-line drugs) is for streptomycin, the reason of which could be high utilization of streptomycin as the main first-line drug in society.

4. OFL and CIP can acceptably be used as two effective antibiotics against first-line drug resistant MOTT strains (however in some cases this effectiveness could be decisive).

5. It seems that effects of the both OFL and CIP drugs against MTB and MOTT strains are approximately the same.

6. Performing drug susceptibility tests of OFL and CIP as two main fluoroquinolones and also other antibiotics are suggested periodically in order to prevent increased incidence of drug resistances and use effective therapy in infections caused by MTB and MOTT.

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