



INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND DEVELOPMENT (IJPRD)

Platform for Pharmaceutical Researches & Innovative Ideas
www.ijprd.com

IN VITRO ANTIMICROBIAL SUSCEPTIBILITY OF MOXIFLOXACIN AND RIFABUTIN AGAINST MYCOBACTERIUM TUBERCULOSIS

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ABSTRACT

Background:

About 5% of all TB cases are multidrug resistant. The new respiratory fluoroquinolones such as moxifloxacin are expected to be effective against strains of Mycobacterium tuberculosis that are resistant to isoniazid and rifampicin.

Methodology:

Aims

The current study was undertaken to evaluate and compare the susceptibility of multidrug-resistant *Mycobacterium tuberculosis* against moxifloxacin and rifabutin

Results

Amongst 53 samples processed, 44 samples were sensitive to Moxifloxacin as compared to 23 samples found sensitive to Rifabutin. Moxifloxacin was found to be statistically superior to Rifabutin ($P < 0.001$).

Conclusion

Moxifloxacin with its differential mechanism of action of inhibiting DNA gyrase may be a better alternative drug in MDR TB as compared to Rifabutin especially when one considers the fact that a considerable degree of cross resistance has been observed between Rifabutin and Rifampicin.

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Key Words

Moxifloxacin, Rifabutin, MDR,
Mycobacterium tuberculosis,
XDR

INTRODUCTION

The incidence of tuberculosis is globally on the rise and India ranks first in terms of total number of cases (2.00 million) amongst all the countries worldwide, the incidence of TB in 2007 being 9.27 million cases^[1].

Pulmonary TB, the most common form of TB, is a highly contagious and life-threatening infection. Moreover, enhanced susceptibility to TB in HIV-infected populations is another serious health problem throughout the world. The alarming increase in the incidence of multidrug resistant TB (MDR-TB) not only in developing countries but industrialized countries as well, during the past decade is a cause for concern. The global resurgence of TB and the rapid emergence of MDR-TB make it imperative to accelerate the search for better anti TB drugs or more effective regimens for efficacious clinical control of TB.

About 5% of all TB cases are multidrug resistant based on data from more than 100 countries collected during the last decade^[2]. Increasing reports of MDR and now, XDR TB (extensively drug-resistant tuberculosis) have stimulated the search for new anti-tubercular agents.

Fluoroquinolones are bactericidal agents which inhibit DNA gyrase and are highly active against *Mycobacterium tuberculosis* including strains resistant to first line agents^[3]. In vitro, animal as well as human studies demonstrated the efficacy of fluoroquinolones^[4].

Moxifloxacin is 8-aminoquinolone which has highest mycobactericidal activity amongst all the quinolones and has shown activity similar to rifampicin in human subjects with pulmonary tuberculosis^[5, 6].

Rifampicin has been considered to be an effective first line anti TB drug which has revolutionized the management of tuberculosis. But rifampicin is associated with several drawbacks including the development of resistance and drug interactions due to induction of the CYP 450 3A4 enzyme.

Rifabutin, a derivative of rifamycin. S. rifabutin is metabolized by human microsomes but not via cytochrome P-450 enzymes^[7].

Rifabutin has become available in the Indian market since 2009. Rifabutin in combination with other drugs is prescribed in regimens for treatment of disseminated *M. avium-intracellulare* infections in HIV patients. When

used alone, it can be an active prophylactic agent against such infection^[8]. While the predominant use of rifabutin is against *Mycobacterium avium intracellulare* infection; there are some reports in literature about the efficacy of rifabutin in MDR TB.

This study was undertaken to evaluate the susceptibility of multidrug-resistant *Mycobacterium tuberculosis* against Moxifloxacin in comparison with Rifabutin.

MATERIALS AND METHODS

Bacterial isolates

Specimens were received from medical out patient clinics and hospitals from Mumbai, Bhuvaneshvar, Delhi, Kanpur and Jaipur. Isolates received at the centralized laboratory were tested for susceptibility to the first line drugs isoniazid and rifampicin.

M. tuberculosis was identified on the basis of microscopic morphology, positive nitrate reduction test and positive pyrazinamidase test^[9].

Method of culture

Three specimens were collected from each patient in wide mouthed containers covered with lids. They were then transported to the laboratory for processing. Specimens containing saliva were discarded. Each specimen was smeared, air dried, fixed and stained with Ziehl- Neelsen (Z-N) reagents using a known acid-fast bacilli (AFB)-stained slide as positive control and a stained slide made of egg albumin as negative control. Results were recorded according to the grading system of the IUATLD (International Union Against Tuberculosis and Lung Disease) as -, scanty, +, ++ or +++ AFB. Then, one of the specimens was cultured onto Lowenstein-Jensen (L-J) slope incubated at 37°C for 6-8 weeks. *Mycobacterium tuberculosis* strain H37RV and sterile L-J medium were used as positive and negative controls, respectively. Growth on L-J slope was restained with Z-N reagents at 2, 4, 6 and 8 weeks of incubation. Thereafter, visible growth was confirmed as *M. tuberculosis* by standard biochemical methods such as positive nitrate reduction test and positive pyrazinamidase test^[9].

Confirmed isolates were collated and stored at 4°C in the refrigerator for subsequent drug susceptibility testing, while those cultures that were contaminated were noted and discarded. Revived *M. tuberculosis*

isolates on L-J slope were tested for drug susceptibility against isoniazid, and rifampicin.

The 53 isolates of *Mycobacterium tuberculosis* that were resistant to both isoniazid and rifampicin were included in the study and drug susceptibility testing to moxifloxacin and rifabutin was performed on Löwenstein-Jensen medium according to the proportion method.

Source of drugs

Antibiotics used in this study i.e. Rifabutin and Moxifloxacin were obtained as pure substances from a leading Indian manufacturer.

Minimum Inhibitory Concentration determination

The antibiotic concentration ranges tested were as follows: Moxifloxacin (0.12 – 0.5 mg/L), Rifabutin (0.05-0.5 mg/L). A standard H37Rv strain was used as a control.

From an uncomplicated bottle with good growth, scrapings were done from all parts of the growth with 0.8 mm loop. This was emulsified in 0.5 ml of sterile distilled water with glass beads in a bijoux bottle and was agitated for one minute. This emulsified suspension was inoculated into different drug containing solid LJ slopes. The media were incubated for 2-3 weeks. The MIC was defined as the lowest concentration showing growth of <1% of that of the initial inoculum on the antibiotic free plate.

Resistant Ratio (RR) was calculated as the ratio of MIC of test strain and MIC of H37Rv.

RESULTS

1.80% samples were pus samples while 98.2% samples were sputum samples. The susceptibility of MDR resistant strains to Moxifloxacin was found to be superior to Rifabutin. Amongst 53 samples processed, 44 samples were sensitive to Moxifloxacin as compared to 23 samples found sensitive to Rifabutin (Table 1).

Table 1 Susceptibility pattern of *M. tuberculosis*

Susceptibility of <i>M. tuberculosis</i>	Moxifloxacin	Rifabutin
Sensitive	44	23
Resistant	9	30

After analysis of the data with Chi square test, Moxifloxacin was found to be statistically superior to Rifabutin ($P < 0.001$).

Discussion

One-third of the global population is infected by *Mycobacterium tuberculosis*, TB remains a major cause of death. TB is particularly severe in parts of Asia and Africa where it is often present in AIDS patients^[10]. The current drawbacks of TB treatment today include the long duration of treatment of 6-9 months and numerous side effects. There is significant concern about the multi-drug-resistant (MDR) strains of TB (0.5 million MDR-TB cases worldwide in 2006)^[10].

The recent global surveillance on drug resistance reveals that there is a clear association between drug resistant tuberculosis including XDR TB and HIV infection.

Drug resistant tuberculosis is associated with high incidence of mortality in HIV patients^[1]. Fluoroquinolones are an important part of second line therapy for multidrug resistant tuberculosis^[11]. Ciprofloxacin has been shown to have early bactericidal activity against *M. tuberculosis*^[12]. Moxifloxacin is the most active fluoroquinolone against *M. tuberculosis*^[5, 10, 13].

The rifamycins, long considered a mainstay of TB treatment, were a major breakthrough in the management of tuberculosis when they were developed in the 1960's^[11]. But rifampicin is associated with several drawbacks such as rapid selection of resistant mutants, hepatotoxicity, a flu-like syndrome (especially at higher doses), potent induction of cytochromes P450 (CYP) and inhibition of hepatic transporters^[10].

Rifabutin is a derivative of rifampicin which was developed to overcome the drawbacks associated with rifampicin. Rifabutin has a reported MIC of 1.0-0.13 µg/ml against MDR strains of *Mycobacterium tuberculosis*^[14].

This study was carried out to explore the pattern of susceptibility of MDR *M. tuberculosis* against Moxifloxacin in comparison with Rifabutin which is frequently used in place of Rifampicin in HIV patients as it does not induce microsomal enzymes^[7]. It is clearly evident from the results that Moxifloxacin is significantly superior to Rifabutin in terms of activity against MDR-

M. tuberculosis. The results are not surprising when one considers the reports of cross resistance between Rifabutin and Rifampicin (72.7%)^[15].

The differential mechanism of bactericidal action of Moxifloxacin which targets the bacterial DNA gyrase and topoisomerase IV ensures that this new fluoroquinolone can be considered to be the drug of choice when treating MDR TB.

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