Original Article

Ind. J. Tub., 1994, 41, 87

IN VITRO ACTIVITY OF CIPROFLOXACIN AND OFLOXACIN AGAINST SOUTH INDIAN ISOLATES OF *MYCOBACTERIUM TUBERCULOSIS*

P. Venkataraman[1], C.N. Paramasivan[2] and R Prabhakar[3]

(Original received on 10.8.93; Revised version received on 16.12.93; Accepted on 9.2.94)

Summary. Mycobacterium tuberculosis isolates from 104 south Indian patients, including 52 sensitive to Streptomycin (S), Isoniazid, (H) and Rifampicin (R), and 52 resistant to SHR/HR were tested for their *in vitro* susceptibility to Ciprofloxacin and Ofloxacin on Lowenstein-Jensen medium. The geometric mean for minimal inhibitory concentration (MIC) of Ciprofloxacin was 2.00 mcg/ml for sensitive strains and 2.17 mcg/ml for resistant strains, the overall mean being 2.08 mcg/ml. Considering Ofloxacin, the MICs for the different categories of strains were again similar, there being no difference between sensitive and resistant strains, the geometric means being 2.00 and 2.05 mcg/ml, respectively.

Introduction

Despite the rapid advances in treatment and the availability of effective short course chemotherapy regimens, tuberculosis continues to be a common disease in the community. Tuberculosis is gaining increasing clinical relevance also because of its association with HIV infection.[1] Treatment for disease due to drug-resistant *M. tuberculosis* is more toxic, more expensive and not as successful as in disease due to drug-sensitive organisms.[2] Effective treatment of patients with multi drug resistant organisms will be greatly facilitated by the development of newer anti-tuberculosis drugs.

Interest in the quinolone group of drugs has grown during the last decade with the development of new derivatives such as Norfloxacin, Pefloxacin, Ofloxacin, Enoxacin, Lomefloxacin and Ciprofloxacin. Of these, Ciprofloxacin is among those with the lowest minimal inhibitory concentration (MIC) against *M. tuberculosis*[3] and is more active than Norfloxacin and Enoxacin. [4,5] A recent study at Tuberculosis Research Centre, Madras has given promising results with Ciprofloxacin on both drug-resistant and drug-sensitive isolates of *M. Tuberculosis, in vitro.*[6]

Ofloxacin has been reported to be active both *in vitro* and *in vivo* against mycobacteria.[7] It was, therefore, proposed to test the *in vitro* activity of Ofloxacin on south Indian isolates of *M. tuberculosis* and compare it with that of Ciprofloxacin. The results of this investigation are reported in this paper.

Material and Methods

Strains : A total of 104 clinical isolates of *M. tuberculosis* from as many patients was tested. These included 52 isolates sensitive to Streptomycin (S), Isoniazid (H) and Rifampicin (R), and 52 resistant to SHR or HR. The standard sensitive strain *M. tuberculosis* $H_{37}Rv$ was also tested.

Drug concentrations : Ciprofloxacin and Ofloxacin were incorporated in Lowenstein Jensen (LJ) medium slopes to give final (pre-inspissation) concentrations of 0.5, 1, 2, 4, 8, 16, 32 and 64 mcg/ml.

Sensitivity testing : A standard suspension (4 mg/ml) of the strains, which were given code numbers to conceal their identity, was inoculated with a 3mm loop on to 2 drug free LJ slopes and one LJ slope each, with the different concentrations of the drugs. All slopes were

1. Senior Technical Officer (Bacteriology);
2. Deputy Director (Bacteriology);.
3. Director, Tuberculosis Research Centre (ICMR), Madras.
Correspondence : Dr. R. Prabhakar, Director, Tuberculosis Research Centre (ICMR), Spurtank Road, Chetput, Madras-600 031.

Table 1. *Minimal inhibitory concentrations of Ciprofloxacin against south Indian isolates of* M. tuberculosis

		No. of strains	MIC* (mcg/ml)			
			0.5	1	2	4
SHR	sensitive	52	1	11	27	13
SHR/HR	resistant	52	1	4	35	12
Total		104	2	15	62	25

*No strain had an MIC > 4 mcg/ml.

Table 2. *Minimal inhibitory concentrations of Ofloxacin against south Indian isolates of M.* tuberculosis

		No. of strains	MIC* (mcg/ml)			
			0.5	1	2	4
SHR	sensitive	52	1	8	33	10
SHR/HR	resistant	52	1	7	33	11
Total		104	2	15	66	21

*No strain had an MIC > 4 mcg/ml.

incubated at 37°C. And, at the end of 4 weeks of incubation, the MIC was determined using the 20-colony end point.[8]

Results

MIC for Ciprofloxacin : The standard strain, *M. tuberculosis* $H_{37}Rv$, tested on three different occasions, gave an MIC of 1 mcg/ml. Considering the strains isolated from patients (Table l), even though 21% (11/52) of SHR sensitive strains had an MIC of 1 mcg/ml, as against 8% (4 out of 52) of SHR/HR resistant strains, the corresponding proportions with MIC of 2 mcg/ml were 52% and 67% respectively. This shift could have been due to experimental variation since the proportions of strains with an MIC of 4 mcg/ml were nearly identical. The geometric mean MICs were 2.00 mcg/ml for sensitive strains and 2.17 mcg/ml for resistant strains, the overall mean being 2.08 mcg/ml.

MIC for Ofloxacin : The MIC of Ofloxacin for *M. tuberculosis* $H_{37}Rv$ was 1 mcg/ml on the two occasions tested. The distributions of the MICs with the two categories of strains were very similar, their being no difference between sensitive and resistant strains, the geometric mean MICs being 2.00 and 2.05 mcg/ml, respectively (Table 2).

Discussion

Although the currently used treatment regimens for pulmonary tuberculosis arc quite effective in patients with drug sensitive organisms, the patients with drug resistant organisms, especially those resistant to Isoniazid and Rifampicin, do not respond well to these regimes.[9] In view of the possibility of an increase in Rifampicin resistance among tuberculosis patients, there is an urgent need for investigations with the newer anti-tuberculosis drugs.[10] These newer drugs include the aminoglycosides, such as Amikacin and Capreomycin, the long acting Rifampicin derivatives, the fluoroquinoloncs and combinations of beta-lactam antibiotics with beta-lactamase inhibitors.[10'11]

Considering first the aminoglycosides, although Amikacin is effective against *M. avium-intracellulare* complex, its activity against *M. tuberculosis* is low and, as such, it might not be effective in the treatment of disease due to *M. tuberculosis.* [11] A recent study from this Centre revealed that 6% of SHR sensitive and 15% of SHR/HR resistant strains could be resistant to Capreomycin.[6] As such, Capreomycin may have only a limited role in the treatment of patients with multiple drug-resistant organisms.

The rifamycin derivatives, Rifapentine and Rifabutin have been reported to be more active than Rifampicin *in vitro* against Rifampicin sensitive strains. Thus, Arioli and others[12] reported a 2 to 10 fold higher activity for Rifapentine than Rifampicin. Recent studies at this Centre revealed that although Rifapentine exhihited a significantly higher activity in Rifampicin sensitive strains, there was complete cross resistance in Rifampicin resistant strains. **On the other hand, Rifabutin was not only more effective than Rifapentine in Rifampicin-sensitive strains but also a small proportion**

(22%) of Rifampicin-resistant strains were susceptible to Rifabutin *in vitro* [13]. Thus, Rifabutin might not only be useful to some extent in the treatment of disease due to Rifampicin-resistant organisms but it could possibly delay the emergence of resistance to the companion drugs making it particularly suitable for intermittent chemotherapy due to its longer half-life,

The introduction of fluoroquinolones has broadened the range of therapeutic tools used against mycobacterial diseases. Being a new class of compounds, there is no cross resistance with the conventional anti-tuberculosis drugs.[9] Of the various derivatives studied, Ciprofloxacin and Ofloxacin have been reported to be most active *in vitro* against *M. tuberculosis.* [3,14,15] **However, the absorption of Ciprofloxacin after oral administration is poor (mean C max 2.4 mcg/ml) with a relatively lower mean half life (4.1 hours) compared to that of Ofloxacin (C max : 11 mcg/ml; t 1/2 : 7 hours).[16,17] Preliminary studies have demonstrated that Ofloxacin is effective against *M. tuberculosis, in vitro* as well as in experimental murine tuberculosis.[9] In a recent uncontrolled study conducted by the Hong Kong Chest Services/British Medical Research Council,[18] it has been reported that Ofloxacin is a relatively better drug in the treatment of drug-resistant pulmonary tuberculosis patients than Rifabutin.**

The present investigation revealed that the *in vitro* activity of Ofloxacin was similar to that of Ciprofloxacin, the overall geometric mean MICs being 2.03 mcg/ml for Ofloxacin and 2.08 mcg/ml for Ciprofloxacin. The earlier reported lower geometric means of MICs from this Centre[6] were perhaps a reflection of laboratory variations in medium preparation, batches of drugs used and doubling dilution concentrations shifting the mean by one step. It may be observed that the mean MIC for Ciprofloxacin is only slightly below the peak serum levels attainable with therapeutic doses of the drug. However, in pulmonary and other tissues the drug may attain levels in excess of those in serum which may be adequate to inhibit growth of strains.[19,20] The mean MIC of Ofloxacin, however, is far below the peak serum level of 11 mcg/ml attainable at normal dosage.[16,17]. Moreover, our results showed that the MICs of both Ciprofloxacin and Ofloxacin were within a narrow range of 1-4 mcg/ml for most of the *M. tuberculosis* strains tested. **Further, since no differences in susceptibility to Ciprofloxacin and Ofloxacin were noted between strains sensitive or resistant to SHR, it can be concluded that there is no cross resistance between these quinolones and standard anti-tuberculosis drugs.** Earlier studies at this Centre also showed no significant differences in the activity of Ciprofloxacin between SHR sensitive and resistant strains.[6]

The present investigation also suggests that Ciprofloxacin and Ofloxacin might be effective in the treatment of patients with multiple drug resistant organisms. But their use in patients could easily lead to the selection of resistant mutants. This, in turn, could mean resistance to the other quinolones, as cross resistance is a well-known phenomenon among the quinolones.[7] **Therefore, these quinolones might be useful in the treatment of multidrug resistant tuberculosis only if used in judicious combination with other drugs to which the strain is sensitive. Thus, the use of Ciprofloxacin or Ofloxacin in the chemotherapy of tuberculosis, including their role in the treatment of failures to standard regimens, can only be assessed after well planned controlled clinical trials.**

Acknowledgments

The authors are grateful to Mrs. Dakshayani Govindhan for technical assistance and Mrs. Jothi Segaran for secretarial assistance.

References

1. Indian Council of Medical Research. HIV infection-current status and future research plans ICMR Bulletin; 1991, **21,** 125.
2. American Thoracic Society. Control of tuberculosis in the United States. Am. Rev. Resp. Dis; 1992, **146,** 1623.
3. Young, L.S., Berlin, O.G.W. and Inderleid, C.B. Activity of Ciprofloxacin and other fluorinated quinolones against mycobacteria. Am. J. Med.; 1987, **82,** (Supl. 4A), 23.
4. Gay J.D., Deyoung D.R. and Roberts G.D. *In vitro* activities of Norfloxacin and Ciprofloxacin against *Mycobacterium tuberculosis, M. avium*

complex, *M. chelonei*, *M. fortuitum* and *M. kansasii* Antimicrob. Agents Chemother.; 1984, **26,** 94.

5. Gaya H. and Chadwick N.V. *In vitro* activity of Ciprofloxacin against mycobacteria. Europ. J. Clin. Microbiol.; 1985, **4,** 345.
6. Venkataraman P., Paramasivan C.N. and Prabhakar R. In *vitro* activity of Capreomycin and Ciprofloxacin against south Indian isolates of *M. tuberculosis.* Ind. J. Tub.; 1993, **40,** 21.
7. Truffot-Pernot, C., Ji. B. and Grosset, J. Activities of Pefloxacin and Ofloxacin against mycobacteria- *In vitro* and mouse experiments. Tubercle; 1991, **72,** 57.
8. Canetti G., Fox W., Khomenko R., Mahler H.T., Menon N.K., Mitchison, D.A., Rist, M. and Smelev, M.A. Advances in the technique of testing mycobacterial drug sensitivity and the use of sensitivity tests in tuberculosis control programmes. Bull. WHO; 1969, **41,** 21.
9. Parenti F. New experimental drugs for the treatment of tuberculosis. Rev. Infect. Dis.; 1989, **11,** (Suppl. 2). 479.
10. Mitchison D.A., Ellard GA. and Grosset J. New antibacterial drugs for the treatment of mycobacterial diseases in man. Brit. Med. Bull.; 1988, **44,** 757.
11. Allen B.W., Mitchison, D.A., Chan Y.C., Yew W.W. and Allen W.G.L. Anicanin in the treatment of pulmonary tuberculosis. Tubercle; 1993, **64,** 111.
12. Arioli V., Berti M., Cameti G., Randisi E. Rossi E., and Scotti R. Antibacterial activity of MDL 473, a new semisynthetic Rifamycin derivative, J. Antibiot. (Tokyo); 1981, **34,** 1026.
13. Venkatarman P., Paramasivan C.N. and Prabhakar R. In vitro activity of Rifampicin, Rifapentine and Rifabutin against south Indian isolates of *Mycobactetium tuberculosis.* Ind. J. Tub.; 1993, **40,** 17.
14. Leysen D.R., Haemers A and Pattyn S.R. Mycobacteria and the new quinolones (Mini review). Antimicrob. Agents Chemother.; 1989, **33,** 1.
15. Van Caekenberghe, D. Comparative *in vitro* activities of ten fluoroquinolones and fusidic acid against *Mycobacterium* spp. J. Antimicrob. Chemother.; 1990, **26,** 381.
16. Hooper D.C. and Wolfson T.S. The fluoroquinolones-pharmacology, clinical uses and toxicities in man (Mini review). Antimicrob. Agents Chemother.; 1985, **28,** 716.
17. Wise R., Lister D., McNulty C.A.M., Griggs, D. and Andrews, J.M. The comparative pharmacokinetics of five quinolones. J. Antimicrob. Chemother.; 1986, **18** (suppl. D), 71.
18. Hong Kong Chest Service/British Medical Research Council. A controlled study of Rifabutin and an uncontrolled study of Ofloxacin in the retreatment of patients with pulmonary tuberculosis resistant to Isoniazid, Streptomycin and Rifampicin. Tubercle and Lung Disease; 1992, **73,** 59.
19. Marinis E. and Legakis N.J. *In vitro* activity of Ciprofloxacin against clinical isolates of mycobacteria resistant to antimycobacterial drugs. J. Antimicrob. Chemother.; 1985, **16,** 527
20. Mehra, P and Aggarwal, K.K. Ciprofloxacin-a new quinolone antibiotic. Ind. J. Clin. Pract.; 1990, **1,** 38.