ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1984, p. 94–96
0066-4804/84/070094-03$02.00/0

Vol. 26, No. 1

NOTES

In Vitro Activities of Norfloxacin and Ciprofloxacin Against *Mycobacterium tuberculosis*, *M. avium* Complex, *M. chelonei*, *M. fortuitum*, and *M. kansasii*

J. DOUGLAS GAY, DONALD R. DEYOUNG, AND GLENN D. ROBERTS*

Section of Clinical Microbiology, Department of Laboratory Medicine, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905

Received 28 November 1983/Accepted 4 April 1984

The activities of ciprofloxacin and norfloxacin against 100 mycobacteria isolates were studied in vitro by the 1% standard proportion method. Ciprofloxacin was more active against *M. tuberculosis* and *M. fortuitum* with MICs of 1.0 and 0.25 μg/ml, respectively, against 90% of isolates; norfloxacin had MICs of 8.0 and 2.0 μg/ml, respectively, against 90% of isolates.

Nalidixic acid and other heterocyclic carbonic acid derivatives have been used primarily in the treatment of urinary tract infections for many years. The compounds of this general group include nalidixic acid, oxolinic acid, pipemidic acid, cinoxacin, and rosoxacin. Two new substances in this series which have been recently synthesized are norfloxacin (6) (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[1-piperazinyl]-3-quinoline carboxylic acid) and ciprofloxacin (1-cyclopropyl-6-fluor-1,4-dihydro-4-oxo-7-[1-piperazinyl]-3-quinoline carbonic acid). In vitro studies (7, 10–13) have shown that norfloxacin is active against a wide variety of gram-positive cocci, gram-negative cocci, and many gram-negative bacilli, including *Haemophilus influenzae*, *Gardnerella vaginalis*, *Pseudomonas aeruginosa*, and members of the *Enterobacteriaceae*. Norfloxacin has been found to be more active than a number of agents against a variety of gram-positive and gram-negative organisms (8, 9). Ciprofloxacin is also effective in vitro against a wide variety of gram-negative and gram-positive organisms. Its activity, however, is greater (1).

The purpose of this study was to determine the in vitro activities of norfloxacin and ciprofloxacin against various species of *Mycobacteria*, including *Mycobacterium tuberculosis*, the *Mycobacterium avium* complex, *Mycobacterium chelonei*, *Mycobacterium fortuitum*, and *Mycobacterium kansasii*.

Until relatively recently, antimicrobial therapy against the rapidly growing mycobacteria (*M. fortuitum* and *M. chelonei*) has included antimycobacterial agents, many of which are not effective against these organisms (20). Within the last several years, many studies have shown a number of antibacterial agents to be active against the rapid growers (2, 3, 15, 18, 19). *M. kansasii* infections usually respond well to intensive triple drug therapy with rifampin and a pair selected from isoniazid, ethambutol, and streptomycin. Infections produced by the *M. avium* complex often pose chemotherapeutic problems, and most isolates have exhibited in vitro resistance to common antimycobacterial agents (4, 14).

Twenty isolates each of *M. tuberculosis*, the *M. avium* complex, *M. chelonei*, *M. fortuitum*, and *M. kansasii* were studied. The organisms were taken from the Mayo Clinic stock culture collection, which included recent clinical isolates. Stock cultures were maintained on Middlebrook 7H10 agar slants (Difco Laboratories, Detroit, Mich.) and were subcultured monthly. The identification of isolates was based on standard biochemical tests (17) and gas-liquid chromatography (16). The ciprofloxacin used in the study was obtained from Miles Pharmaceuticals, Div. of Miles Laboratories, Inc., West Haven, Conn. Norfloxacin was obtained from Merck Sharp & Dohme, Rahway, N.J. A working solution of ciprofloxacin was prepared in sterile distilled water; norfloxacin was prepared in 0.1 mol of NaOH per ml. Appropriate concentrations of each were added to melted Middlebrook 7H11 agar before testing.

The standard 1% proportion method was used to determine the MICs (17), and the concentrations of antibiotics used were $\log_2$ dilution steps within the range of 0.25 to 16 μg/ml. After a suspension of each isolate was made equivalent to a McFarland no. 1 standard, an additional 10^{-6} dilution was performed. Three drops of this suspension containing approximately 100 to 150 CFU were placed onto each of four quadrants of a plate containing Middlebrook 7H10 agar, pH 6.6 (Difco Laboratories). Each plate contained one quadrant with no antimicrobial agent; the remaining three quadrants contained the appropriate concentration of the agent being tested. Cultures were incubated at 35°C in an atmosphere of 5 to 7% CO_2. Results for the rapidly growing mycobacteria were recorded after 7 days, and those of the other species were recorded after 15 days. The MIC was defined as the antibiotic concentration of the quadrant which yielded a colony count of 1% or less than that observed on the antimycobacterial agent-free quadrant.

The conventional antimycobacterial drugs isoniazid, streptomycin, ethambutol, and rifampin were also tested. MICs of >5.0, >10.0, >15.0, and >10.0 μg/ml, respectively, were considered to indicate resistance to these drugs.

Table 1 presents the ranges of MICs of ciprofloxacin and norfloxacin for the 100 isolates of *Mycobacteria*. The range of MICs of each drug for each species varied widely. However, the MICs inhibiting 50 and 90% of the isolates (MIC_{50} and MIC_{90}, respectively) were significantly lower for ciprofloxacin than for norfloxacin with each species tested.

* Corresponding author.

TABLE 1. Susceptibility of 100 strains of five species of mycobacteria to norfloxacin and ciprofloxacin

Organism[a]	MIC (μg/ml)					
	Ciprofloxacin			Norfloxacin		
	Range	50%	90%	Range	50%	90%
M. tuberculosis	0.25–1	0.5	1	2–8	4	8
M. avium complex	0.5–>16	2	16	2–>16	16	>16
M. chelonei	0.25–16	1	8	0.5–>16	16	>16
M. fortuitum	0.25–8	0.25	0.25	0.25–>16	0.5	2
M. kansasii	1–4	2	4	8–>16	16	>16

[a] Twenty isolates of each species were tested.

M. fortuitum and *M. tuberculosis* were most susceptible to both agents, whereas the isolates of the *M. avium* complex, *M. kansasii*, and *M. chelonei* were relatively resistant. Results, however, were strain dependent.

Table 2 presents the available results on the susceptibilities of the isolates to standard antimycobacterial agents; results were not available for all isolates. As might be expected, the *M. avium* complex, *M. fortuitum*, and *M. chelonei* were relatively resistant to these agents, whereas *M. tuberculosis* and *M. kansasii* were much more susceptible.

These data suggest that ciprofloxacin and norfloxacin may be active against clinically important species of mycobacteria, particularly *M. tuberculosis* and *M. fortuitum*. Since *M. fortuitum* is usually resistant to the standard antimycobacterial agents, alternative therapy has been sought. A number of relatively nontoxic agents have been found to be effective against this organism. Based on levels achievable in blood, some of the most active agents against *M. fortuitum* include amikacin, sulfamethoxazole, sulfamethoxazole-trimethoprim, doxycycline, and minocycline (15). In the United States, fortunately, most newly discovered cases of *M. tuberculosis* are susceptible to the major drugs, including isoniazid, streptomycin, ethambutol, and rifampin.

The expected peak levels of ciprofloxacin in plasma after oral doses of 250 and 500 mg are 0.815 ± 0.12 and 1.58 ± 0.11 mg/liter, respectively (R. Ziegler, K.-H. Graefe, W. Wingender, W. Gau, H.-J. Zeiler, U. Neitz, and P. Schacht, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 851, 1983). As noted in Table 1, these levels would appear to exceed both the MIC_{50} and the MIC_{90} of ciprofloxacin against *M. fortuitum*. Ciprofloxacin appears much less active against *M. chelonei*. The achievable ciprofloxacin level would be expected to exceed the MIC_{90} for *M. tuberculosis*.

The achievable peak level of norfloxacin in plasma after an oral dose of 400 mg is 1.35 μg/ml (5). As noted in Table 1, this expected level in plasma would exceed the MIC_{50} for *M. fortuitum*. However, this level would not apparently be effective against the other species.

TABLE 2. Susceptibility results of five species of mycobacteria to standard antimycobacterial agents

Organism	Isolates susceptible/isolates tested (%)[a]			
	Isoniazid	Streptomycin	Ethambutol	Rifampin
M. tuberculosis	20/20 (100)	19/20 (95)	20/20 (100)	20/20 (100)
M. avium complex	2/19 (10.5)	1/19 (5.2)	11/19 (57.9)	4/19 (21)
M. chelonei	0/15 (0)	2/15 (13.3)	3/15 (20)	4/15 (26.7)
M. fortuitum	3/16 (18.7)	1/16 (6.3)	3/16 (18.8)	4/16 (25)
M. kansasii	18/20 (90)	19/20 (95)	16/20 (80)	20/20 (100)

[a] Susceptibility was determined as susceptibility to the following MICs of the drugs: isoniazid, ≤5.0 μg/ml; streptomycin and rifampin, ≤10.0 μg/ml; ethambutol, ≤15.0 μg/ml.

In this study, norfloxacin and ciprofloxacin were most active against *M. tuberculosis* and *M. fortuitum*. Ciprofloxacin exhibited greater activity than norfloxacin, as has been shown for gram-positive and gram-negative bacteria in another in vitro study (1). Ciprofloxacin may be useful in the treatment of mycobacterial infections, particularly those caused by *M. tuberculosis*, *M. fortuitum*, and some isolates of *M. chelonei*. Ciprofloxacin may serve as an alternative agent for the treatment of tuberculosis since most common antimycobacterial agents are effective. In addition, it may be useful in cases of infection caused by *M. fortuitum* and *M. chelonei*, the organisms which are most often resistant to most antimicrobial agents used for treatment. Norfloxacin may also be useful for the treatment of infection caused by some isolates of *M. fortuitum*; however, all other species tested had MICs higher than achievable levels in serum. The potential efficacy of these drugs in the treatment of mycobacterial infections requires additional in vitro and clinical studies.

Thanks are extended to Hans Gadebusch for supplying norfloxacin and to Barbara Painter for supplying ciprofloxacin for this study.

LITERATURE CITED

1. **Bauerfeind, A., and C. Petermuller.** 1983. *In vitro* activity of ciprofloxacin, norfloxacin, and nalidixic acid. Eur. J. Clin. Microbiol. **2**:111–115.
2. **Cynamon, M. H., and A. Patapow.** 1981. In vitro susceptibility of *Mycobacterium fortuitum* to cefoxitin. Antimicrob. Agents Chemother. **19**:205–207.
3. **Dalovisio, J. R., and G. A. Pankey.** 1978. In vitro susceptibility of *Mycobacterium fortuitum* and *Mycobacterium chelonei* to amikacin. J. Infect. Dis. **137**:318–321.
4. **Dutt, A. K., and W. W. Stead.** 1979. Long term results of medical treatment in *Mycobacterium intracellulare* infection. Am. J. Med. **67**:449–453.
5. **Eandi, M., I. Viano, F. DiNola, L. Leone, and E. Geniazzani.** 1983. Pharmakokinetics of norfloxacin in healthy volunteers and patients with renal and hepatic damage. Eur. J. Clin. Microbiol. **2**:253–259.
6. **Gadebusch, H. H., L. R. Koupal, E. Celozzi, D. L. Shungu, J. Bland, B. Weissberger, B. A. Pelak, E. Fisch, S. K. Chung, and J. Huber.** 1982. Norfloxacin (MK-0366, AM-715), a new orally absorbed synthetic compound for the treatment of bacterial infections, p. 351–353. *In* P. Periti and G. G. Grassi (ed.), Current chemotherapy and immunotherapy. Proceedings of the 12th International Congress of Chemotherapy, vol. 1. American Society for Microbiology, Washington, D.C.
7. **Gadebusch, H. H., D. L. Shungu, E. Weinberg, and S. K. Chung.** 1982. Comparison of the antibacterial activity of norfloxacin (MK-366, AM-715), a new organic acid with that of other orally absorbed chemotherapeutic agents. Infection **10**:41–44.
8. **Haase, D., B. Urias, G. Harding, and A. Ronald.** 1983. Comparative *in vitro* activity of norfloxacin against urinary tract pathogens. Eur. J. Clin. Microbiol. **2**:235–241.
9. **Khan, M. Y., R. P. Gruninger, S. M. Nelson, and R. E. Klicker.** 1982. Comparative in vitro activity of norfloxacin (MK-366) and ten other oral antimicrobial agents against urinary bacterial isolates. Antimicrob. Agents Chemother. **21**:848–851.
10. **Khan, M. Y., Y. Siddiqui, and R. P. Gruninger.** 1981. Comparative in vitro activity of MK-0366 and other selected oral antimicrobial agents against *Neisseria gonorrhoeae*. Antimicrob. Agents Chemother. **20**:265–266.
11. **King, A., C. Warren, K. Shannon, and I. Phillips.** 1982. In vitro antibacterial activity of norfloxacin (MK-0366). Antimicrob. Agents Chemother. **21**:604–607.
12. **Neu, H. C., and P. Lathavikul.** 1982. In vitro activity of norfloxacin, a quinolinecarboxylic acid, compared with that of

β-lactams, aminoglycosides, and trimethoprim. Antimicrob. Agents Chemother. **22**:23–27.
13. **Newson, S. W. B., J. Mathews, M. Amphlett, and R. E. Warren.** 1982. Norfloxacin and the antibacterial gamma pyridone beta carboxylic acids. J. Antimicrob. Chemother. **10**:25–30.
14. **Rosezweig, D. Y.** 1979. Pulmonary mycobacterial infections due to *Mycobacterium intracellulare-avium* complex. Chest **75**:115–119.
15. **Swenson, J. M., C. Thornsberry, and V. A. Silcox.** 1982. Rapidly growing mycobacteria: testing of susceptibility to 34 antimicrobial agents by broth microdilution. Antimicrob. Agents Chemother. **22**:186–192.
16. **Tisdall, P. A., G. D. Roberts, and J. P. Anhalt.** 1979. Identification of clinical isolates of mycobacteria with gas-liquid chromatography alone. J. Clin. Microbiol. **10**:506–514.
17. **Vestal, A. L.** 1975. Procedures for the isolation and identification of mycobacteria, p. 41–115. U.S. Department of Health, Education and Welfare publication no. (CDC) 75-8230. Centers for Disease Control, Atlanta.
18. **Wallace, R. J., Jr., J. R. Dalovisio, and G. A. Pankey.** 1979. Disk diffusion testing of susceptibility of *Mycobacterium fortuitum* and *Mycobacterium chelonei* to antibacterial agents. Antimicrob. Agents Chemother. **16**:611–614.
19. **Wallace, R. J., Jr., D. B. Jones, and K. Wiss.** 1981. Sulfonamide activity against *Mycobacterium fortuitum* and *Mycobacterium chelonei*. Rev. Infect. Dis. **3**:898–904.
20. **Wolinsky, E.** 1979. Nontuberculous mycobacteria and associated diseases. Am. Rev. Respir. Dis. **119**:107–159.