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In Vitro Susceptibility of *Mycobacterium avium* Complex to the New Fluoroquinolone Sparfloxacin (CI-978; AT-4140) and Comparison with Ciprofloxacin

DAVID M. YAJKO,* CYNTHIA A. SANDERS, PATRICIA S. NASSOS, AND W. KEITH HADLEY

Department of Laboratory Medicine, University of California, San Francisco General Hospital Medical Center, San Francisco, California 94110

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We tested the activity of the new fluoroquinolone sparfloxacin (CI-978; AT 4140) against 30 strains of *Mycobacterium avium* complex (MAC) isolated from patients with acquired immune deficiency syndrome. MICs of sparfloxacin (range, ≤0.06 to 4 μg/ml) were lower than MICs of ciprofloxacin for all 30 strains, and MBCs for acid-fast bacteria were lower for 28 of the 30 strains. In synergism experiments using 10 strains of MAC, fractional inhibitory concentration indices revealed that the combination of sparfloxacin plus ethambutol was synergistic against 9 strains, and the three-drug combination of sparfloxacin plus ethambutol plus rifampin was synergistic against all strains. In the absence of ethambutol, the combination of sparfloxacin plus rifampin appeared to be antagonistic against three of the MAC strains.

Some members of the fluoroquinolone class of antimicrobial agents show in vitro activity against *Mycobacterium avium* complex (MAC) (2, 4, 5). Ciprofloxacin and WIN 57273 are among the most active compounds in this group of agents (2, 4), with ciprofloxacin showing inhibitory activity against approximately 30% of MAC strains (11). Sparfloxacin (CI-978; AT-4140) is a new agent in the fluoroquinolone class of antimicrobial agents (7, 8, 10). To examine the in vitro activity of this new agent against MAC, we tested sparfloxacin against 30 strains of MAC isolated at San Francisco General Hospital from patients with acquired immune deficiency syndrome (AIDS) and compared this activity with that of ciprofloxacin. In addition, since ciprofloxacin has been shown to act synergistically with ethambutol and rifampin (11), synergism experiments were performed using sparfloxacin in combination with ethambutol and/or rifampin against 10 of the MAC strains.

Sparfloxacin was provided by Parke-Davis (Ann Arbor, Mich.) and was tested over a range of 0.06 to 4 μg/ml. The drug was prepared by adding 10 mg of powder to 0.5 ml of 95% ethanol, then adding 5 ml of sterile distilled water and 2 to 3 drops of 1 N NaOH, and mixing until the powder was dissolved. The volume was brought up to 10 ml with water to achieve a final concentration of 1 mg/ml. Dilutions of the stock solution were prepared in 7HSF broth, a broth medium that is the equivalent of 7H11 agar (12). Solutions of ciprofloxacin, ethambutol, and rifampin were prepared according to manufacturers' recommendations and diluted in 7HSF broth.

The 30 strains of MAC used in this study were stored at −70°C and were passaged four to eight times subsequent to their primary isolation. MICs and MBCs for acid-fast bacteria [MBC(AFB)] were determined by using 2 ml of 7HSF broth and ca. 3×10^5 CFU of actively growing MAC per ml, as previously described (12, 13). Colony counts were done on the initial inoculum in order to permit measurement of the killing effect of antimicrobial agents. Tubes were scored for turbidity after 7 days of incubation at 35°C. Tubes showing no turbidity were subcultured (0.1 ml of a 1:10 dilution) to 7H10 agar to determine the MBC(AFB) (13). The MBC(AFB) is defined as the lowest concentration of drug that kills >99% of the initial inoculum (13).

Table 1 shows a comparison of the MICs and MBC(AFB)s of sparfloxacin and ciprofloxacin against 30 strains of MAC. The MIC of sparfloxacin was lower than the MIC of ciprofloxacin for each of the 30 strains, while the MBC(AFB) of sparfloxacin was lower than the corresponding MBC(AFB) of ciprofloxacin for 28 of the 30 strains. The table also shows that a correlation exists between strains for which ciprofloxacin MICs were low (seven strains for which MICs were ≤0.5) and strains for which sparfloxacin MICs were low (same seven strains; MICs, ≤0.12). For sparfloxacin, the MICs for 50 and 90% of the strains (MIC_{50} and MIC_{90}, respectively) were 1 and 2 μg/ml, respectively, while the MBC_{50} and MBC_{90} were 2 and 4 μg/ml, respectively. For ciprofloxacin, the MIC_{50}, MIC_{90}, MBC_{50}, and MBC_{90} were all >4 μg/ml. The MIC_{50}s and MIC_{90}s obtained here for sparfloxacin are similar to those reported recently for WIN 57273 (4).

On the basis of the achievable concentrations of sparfloxacin (ca. 1 μg/ml) (6) and ciprofloxacin (ca. 3 μg/ml) in serum, 70% of the MAC strains could be classified as susceptible to inhibition by sparfloxacin and 33% could be classified as susceptible to inhibition by ciprofloxacin. Similarly, by using the MBC(AFB)s, 43% of the strains could be classified as killed by sparfloxacin and 30% could be classified as killed by ciprofloxacin on the basis of the achievable concentration of each drug in serum.

Synergism tests were performed in 7HSF broth by the method of Hallander et al. (3). The fractional inhibitory concentration (FIC) and the fractional bactericidal concentration (FBC) indices were determined for each drug combination, as previously described (11). Briefly, drugs were added to tubes of 7HSF broth so that each drug was present at its MIC. Serial twofold dilutions of the drug combinations were prepared in broth, and the tubes were inoculated with MAC at a concentration of ca. 3×10^5 CFU/ml. Tubes were examined for turbidity after 7 days of incubation at 35°C. Tubes showing no turbidity were subcultured to determine

* Corresponding author.

TABLE 1. MICs and MBC(AFB)s of sparfloxacin and ciprofloxacin against AIDS-derived MAC strains

Strain no.	Sparfloxacin		Ciprofloxacin	
	MIC (μg/ml)	MBC(AFB) (μg/ml)[a]	MIC (μg/ml)	MBC(AFB) (μg/ml)
1	1	4	>4	>4
2	2	2	4	>4
3	1	4	>4	>4
4	≤0.06	0.12	0.5	0.5
5	1	2	>4	>4
6	2	>4	>4	>4
7	0.12	0.25	0.5	2
8	2	4	>4	>4
9	2	4	>4	>4
10	0.12	0.25	1	1
11	≤0.06	0.5	0.25	0.25
12	0.12	0.25	0.5	2
13	2	2	>4	>4
14	1	2	2	4
15	≤0.06	0.12	0.25	0.5
16	0.5	1	>4	>4
17	2	2	>4	>4
18	1	2	>4	>4
19	1	2	4	>4
20	2	4	>4	>4
21	0.5	0.5	2	2
22	0.12	0.25	0.5	0.5
23	0.5	1	4	>4
24	2	4	>4	>4
25	0.12	0.25	0.25	0.5
26	1	1	>4	>4
27	1	2	>4	>4
28	4	4	>4	>4
29	1	1	>4	>4
30	1	2	4	>4

[a] Defined as the lowest concentration that kills >99% of the initial inoculum.

survival, as described above. The FIC was calculated as the MIC of drug in combination divided by the MIC of drug alone, and the FBC was calculated as the MBC(AFB) of drug in combination divided by the MBC(AFB) of drug alone (3). The FIC index is the sum of the FIC for each drug in combination, and the FBC index is the sum of the FBC of each drug in combination (3). Synergism was defined as an FIC or FBC index of ≤0.5 for combinations involving two drugs (3, 11), and an FIC or FBC index of ≤0.75 for combinations of three drugs (11), based on the definition of Berenbaum (1).

FIC and FBC indices of sparfloxacin in combination with ethambutol or rifampin or both are shown in Table 2. The results show that the combination of ethambutol plus sparfloxacin was synergistic against 9 of 10 strains of MAC. The combination of sparfloxacin plus rifampin was synergistic against two strains, additive (ΣFIC = 1.0) against five strains, and apparently antagonistic (ΣFIC ≥2.0) against three strains. In vitro antagonism against MAC by the combination of ciprofloxacin plus rifampin has previously been reported (11). This antagonism could be overcome by the addition of ethambutol to the two-drug combination (11). In the present study, the three-drug combination of sparfloxacin plus ethambutol plus rifampin showed synergistic activity (ΣFIC, ≤0.75) against all 10 strains of MAC tested. Killing synergism (ΣFBC, ≤0.75) occurred against 8 of 10 strains of MAC when this three-drug combination was used.

This study demonstrates the following: (i) sparfloxacin is more active in vitro than ciprofloxacin against AIDS-derived strains of MAC; (ii) sparfloxacin acts synergistically with ethambutol against most (90%) strains of MAC and against all strains when it is combined with ethambutol and rifampin; and (iii) as was found with ciprofloxacin, it appears that sparfloxacin can be antagonistic against MAC when combined with rifampin, but this antagonism can be overcome by the addition of ethambutol. Considering the poor response of patients with AIDS to treatment with other antimicrobial agents (9), sparfloxacin should be tested further for possible use in the treatment of *M. avium* complex infections in patients with AIDS.

TABLE 2. FIC and FBC indices of sparfloxacin in combination with other drugs[a]

Strain no.	Sparfloxacin + rifampin		Sparfloxacin + ethambutol		Sparfloxacin + rifampin + ethambutol	
	ΣFIC	ΣFBC	ΣFIC	ΣFBC	ΣFIC	ΣFBC
1	1.0	0.38	0.5	0.19	0.38	0.56
2	0.5	1.0	0.5	0.28	0.19	0.75
3	0.5	0.75	0.25	0.31	0.19	0.11
4	1.0	1.0	0.5	0.38	0.38	0.16
5	1.0	0.75	0.5	0.5	0.75	0.31
6	2.0	1.0	0.5	0.38	0.75	<0.75
7	1.0	1.0	0.5	0.38	0.75	0.31
8	2.0	0.75	0.5	0.38	0.38	0.88
9	>2.0	>1.0	1.0	>1.0	0.75	0.22
10	1.0	>1.0	0.5	0.75	0.38	1.0

[a] FIC = MIC of drug in combination/MIC of drug alone; ΣFIC = sum of the FICs for each drug in the combination; FBC = MBC of drug in combination/MBC of drug alone; ΣFBC = sum of the FBCs for each drug in the combination.

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