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NOTES

In Vitro Activity of *S*-Ofloxacin

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S-Ofloxacin, the optically active form of ofloxacin, was twice as active as the *S,R* mixture of ofloxacin against members of the family *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and gram-positive species. Of the *Enterobacteriaceae*, 90% were inhibited by ≤1 μg/ml and 90% of *Staphylococcus aureus* and *Streptococcus pyogenes* isolates were inhibited by 0.5 μg/ml. *Bacteroides fragilis* was inhibited by 4 μg/ml. Organisms resistant to ofloxacin were resistant to *S*-ofloxacin. Like ofloxacin activity, the activity of *S*-ofloxacin was reduced by Mg^{2+} and by acid pH. Spontaneous mutational resistance to *S*-ofloxacin was similar to that to ofloxacin.

There has been continued interest in improving the antibacterial activity of the new quinolone antimicrobial agents. Ofloxacin exists as two optically active forms because of the asymmetric center at position C-3 of the oxazine ring that links the N-1 and C-8 positions. Preliminary studies have shown that the *S* isomer of ofloxacin was more active than the mixture (3). Similar results have been shown for the *S* isomer of S-25930 (2, 9). We wished to determine the activity of the *S* isomer compared with that of ofloxacin against clinical isolates, including strains collected from patients treated with ofloxacin or ciprofloxacin.

S-Ofloxacin and ofloxacin were gifts from Ortho Pharmaceutical Corp., Raritan, N.J. Organisms were isolates from patients seen at The Columbia-Presbyterian Medical Center in New York City and included isolates from patients treated in clinical evaluations of ofloxacin and ciprofloxacin. MICs were determined by the National Committee for Clinical Laboratory Standards procedures (7) with Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.). GC agar was supplemented with lysed sheep blood and IsoVitaleX for *Neisseria*, *Haemophilus*, and *Branhamella* species. Brucella agar supplemented with 5% sheep blood, hemin, and vitamin K was used for anaerobic species. Incubation was for 48 h at 35°C in GasPak jars (BBL). Bactericidal activity was determined as outlined by National Committee for Clinical Laboratory Standards (6). The effect of growth conditions was as previously described (4). The selection of resistant isolates and spontaneous mutants was as previously described (1). Porin mutants used were a gift of H. Nakaido.

The overall activity of *S*-ofloxacin compared with that of ofloxacin is given in Table 1. The MIC of *S*-ofloxacin at which 90% of the members of the family *Enterobacteriaceae* were inhibited with 1 μg/ml. This was twofold less than the MIC of ofloxacin. In virtually every instance, the MIC of *S*-ofloxacin was, on repeat testing, 1 dilution lower than that of ofloxacin. For example, if the MIC for *Escherichia coli* was 0.12 μg of ofloxacin per ml, the MIC of *S*-ofloxacin was 0.06 μg/ml. This was true for *Citrobacter*, *Enterobacter*, *Morganella*, and *Proteus* isolates as well. The activity of *S*-ofloxacin against *Pseudomonas aeruginosa* also was twofold lower than that of ofloxacin, with 90% of isolates, which included ceftazidime- and imipenem-resistant isolates, inhibited by 4 μg/ml.

Against gram-positive species, the MIC for 90% of strains tested ranged from 0.25 to 2 μg/ml, with *Streptococcus pyogenes* and *Streptococcus pneumoniae* inhibited by 1 μg/ml, as was *Enterococcus faecalis*. *S*-Ofloxacin was also twofold more active than ofloxacin against the *Bacteroides* and *Clostridium* species tested.

The activities of *S*-ofloxacin were similar in Mueller-Hinton, brain heart infusion, and nutrient agars. The addition of 50% human serum did not alter the activity. The addition of Mg^{2+} (Table 2) caused an increase in MICs and MBCs, but the drug remained twofold more active than ofloxacin. The addition of calcium had no effect. Activity of *S*-ofloxacin was also decreased for *E. coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *P. aeruginosa* when assays were performed with urine (pH 5.5) as medium, and there was a four- to eightfold increase in MICs and MBCs at pH 5.5 over those at pH 7.4, but the activity of *S*-ofloxacin was always superior to that of ofloxacin (data not shown).

The frequency of development of spontaneous resistance to *S*-ofloxacin to fourfold the MIC was 10^{-9} to 10^{-10} for two isolates each of *E. coli*, *K. pneumoniae*, *S. marcescens*, and *P. aeruginosa*. The MICs of *S*-ofloxacin were increased for an *E. coli* isolate selected for resistance to ofloxacin by repeated subculture and for a *P. aeruginosa* strain isolated from a patient with cystic fibrosis who had been treated with ofloxacin (Table 3). As before, MICs of *S*-ofloxacin were twofold lower than those of ofloxacin. The *S*-ofloxacin MIC for *E. coli* mutants which were $OmpF^-$ was 0.06 μg/ml, compared with an ofloxacin MIC of 0.12 μg/ml. The MICs of both drugs were twofold lower for the parent *E. coli* $OmpF^+$ $OmpC^+$ strains.

These studies confirm and extend earlier observations about the activity of the isomer of ofloxacin (3, 8). The difference in the activity has been shown to be related to the DNA gyrase activity (5). The *S* isomer of compound S-

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TABLE 1. Comparative activity of ofloxacin and *S*-ofloxacin

Organism (no. tested)	Agent	MIC (μg/ml)[a]		
		Range	50%	90%
Escherichia coli (30)	Ofloxacin	0.06–0.12	0.06	0.12
	S-Ofloxacin	0.015–0.12	0.03	0.06
Klebsiella pneumoniae (20)	Ofloxacin	0.12–2	0.25	0.25
	S-Ofloxacin	0.06–1	0.12	0.25
Enterobacter cloacae (15)	Ofloxacin	0.06–0.5	0.12	0.25
	S-Ofloxacin	0.06–0.25	0.12	0.25
Enterobacter aerogenes (15)	Ofloxacin	0.06–0.25	0.25	0.25
	S-Ofloxacin	0.03–0.25	0.12	0.25
Citrobacter freundii (20)	Ofloxacin	0.12–2	0.25	2
	S-Ofloxacin	0.06–1	0.25	1
Citrobacter diversus (22)	Ofloxacin	0.06	0.06	0.06
	S-Ofloxacin	0.03–0.06	0.03	0.03
Proteus mirabilis (20)	Ofloxacin	0.12–2	0.25	0.5
	S-Ofloxacin	0.06–1	0.12	0.25
Morganella morganii (20)	Ofloxacin	0.03–0.25	0.06	0.12
	S-Ofloxacin	0.015–0.12	0.03	0.12
Proteus vulgaris (20)	Ofloxacin	0.06–4	0.12	1
	S-Ofloxacin	0.06–2	0.06	0.5
Providencia rettgeri (10)	Ofloxacin	0.06–4	1	2
	S-Ofloxacin	0.06–2	0.5	1
Providencia stuartii (20)	Ofloxacin	0.06–8	0.5	2
	S-Ofloxacin	0.03–8	0.25	1
Serratia marcescens (28)	Ofloxacin	0.25–4	0.5	0.5
	S-Ofloxacin	0.12–2	0.25	0.25
Pseudomonas aeruginosa (30)	Ofloxacin	1–64	4	8
	S-Ofloxacin	0.5–16	2	4
Pseudomonas aeruginosa, ceftazidime resistant (30)	Ofloxacin	1–64	4	8
	S-Ofloxacin	0.5–16	2	4
Pseudomonas cepacia (18)	Ofloxacin	0.12–64	2	8
	S-Ofloxacin	0.03–16	0.5	4
Acinetobacter spp. (18)	Ofloxacin	0.12–1	0.5	1
	S-Ofloxacin	0.06–0.5	0.25	0.5
Staphylococcus aureus, methicillin resistant (25)	Ofloxacin	0.5–2	0.5	2
	S-Ofloxacin	0.12–1	0.25	0.5
Staphylococcus aureus, methicillin susceptible (25)	Ofloxacin	0.25–1	0.5	1
	S-Ofloxacin	0.12–0.5	0.25	0.5
Staphylococcus epidermidis, methicillin resistant (14)	Ofloxacin	0.25–1	0.5	1
	S-Ofloxacin	0.12–0.5	0.25	0.25
Staphylococcus epidermidis, methicillin susceptible (12)	Ofloxacin	0.25–2	0.5	2
	S-Ofloxacin	0.25–1	0.25	1
Streptococcus pyogenes group A (20)	Ofloxacin	0.5–2	1	1
	S-Ofloxacin	0.25–0.5	0.5	0.5
Streptococcus agalactiae (20)	Ofloxacin	1–4	1	4
	S-Ofloxacin	0.5–2	1	2
Group G streptococci (10)	Ofloxacin	0.5–2	1	1
	S-Ofloxacin	0.25–0.5	0.5	0.5
Group C and F streptococci (14)	Ofloxacin	1–4	1	2
	S-Ofloxacin	0.5–2	0.5	1
Enterococcus faecalis (31)	Ofloxacin	2–4	2	4
	S-Ofloxacin	0.5–1	0.5	1
Streptococcus pneumoniae (21)	Ofloxacin	1–2	2	2
	S-Ofloxacin	0.5–1	1	1
Listeria monocytogenes (14)	Ofloxacin	1–2	2	2
	S-Ofloxacin	0.5–1	1	1
Haemophilus influenzae (14)	Ofloxacin	0.015–0.03	0.03	0.03
	S-Ofloxacin	<0.015–0.015	0.015	0.015
Branhamella catarrhalis (8)	Ofloxacin	0.03–0.25	0.25	
	S-Ofloxacin	<0.015–0.06	0.06	
Bacteroides fragilis (18)	Ofloxacin	2–16	4	8
	S-Ofloxacin	1–8	2	4
Clostridium perfringens (15)	Ofloxacin	0.25–0.5	0.05	0.5
	S-Ofloxacin	0.12–0.25	0.25	0.25
Peptostreptococci (4)	Ofloxacin	$1, 2_2, 4$		
	S-Ofloxacin	$0.5_2, 1, 2$		
Clostridium spp. (4)	Ofloxacin	0.25, 0.5, 2, 8		
	S-Ofloxacin	0.12, 0.25, 0.5, 4		
Propionibacterium spp. (3)	Ofloxacin	$1_2, 2$		
	S-Ofloxacin	$0.25_2, 0.5$		
Corynebacterium jekeium (5)	Ofloxacin	2_5		
	S-Ofloxacin	1_5		

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively. When fewer than six isolates of a species were tested, the MICs for individual isolates are given; the number of isolates with a given MIC is indicated by an inferior number.

TABLE 2. Effect of magnesium and calcium on the activities of ofloxacin and *S*-ofloxacin

Organism	Agent	MIC/MBC (μg/ml) on MHB[a]:		
		Alone	+4.5 mM Ca^{2+}	+9 mM Mg^{2+}
Escherichia coli 5800	Ofloxacin	0.03/0.03	0.06/0.06	0.25/0.5
	S-Ofloxacin	0.03/0.03	0.03/0.03	0.12/0.12
Klebsiella pneumoniae 8708	Ofloxacin	0.03/0.06	0.12/0.12	0.5/2
	S-Ofloxacin	0.03/0.03	0.06/0.06	0.25/0.5
Enterobacter cloacae 80	Ofloxacin	0.5/0.5	0.5/0.5	1/1
	S-Ofloxacin	0.25/0.25	0.12/0.12	0.5/1
Serratia marcescens 86	Ofloxacin	0.12/0.12	0.25/0.5	1/4
	S-Ofloxacin	0.12/0.12	0.12/0.5	0.5/1
Pseudomonas aeruginosa 158	Ofloxacin	1/4	1/4	4/32
	S-Ofloxacin	0.5/4	0.5/4	2/8

[a] MHB, Mueller-Hinton broth.

TABLE 3. Comparison of the cross-resistance to ofloxacin and *S*-ofloxacin

Organism	Cross-resistance (MIC, μg/ml) to:	
	Ofloxacin	*S*-ofloxacin
E. coli 5039[a]		
Parent	0.12	0.06
Mutant	0.5	0.25
Mutant	2	1
P. aeruginosa 161[b]		
Parent	1	0.5
Mutant	16	8
Mutant	16	8

[a] Mutants were selected by repeated passage for 14 days in the presence of increasing concentrations of ofloxacin.
[b] Mutants were isolated during therapy of a patient with cystic fibrosis.

25930, which also is a tricyclic quinolone, is more active than the *R* isomer (2, 9).

The MICs in this study for gram-positive species are similar to those recently reported by Une et al. (8), but we found considerably lower MICs for 90% of members of the *Enterobacteriaceae* and for *P. aeruginosa* than did those investigators. Although the isolates used in this study had been collected over the past 4 years during therapy of patients with quinolones, we had few resistant organisms

except among *P. aeruginosa* isolates. The extended use of quinolones in Japan or selection of highly resistant isolates in the study by Une et al. (8) may account for the differences.

S-Ofloxacin has been reported to be well absorbed in animals and humans, as is ofloxacin (8). It is possible that *S*-ofloxacin can be a useful compound, clinically permitting use of lower doses because of its twofold-greater activity or permitting treatment of infections with organisms for which the ofloxacin MICs are 2 to 4 μg/ml.

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