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In Vitro Antibacterial Activity of Norfloxacin (MK-0366)

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The in vitro activity of norfloxacin (MK-0366) compared with that of β-lactam antibiotics and, where appropriate, of gentamicin or metronidazole was assessed against recent clinical isolates of common bacteria. The compound was highly active against most enterobacteria (minimal inhibitory concentrations [MICs], 0.008 to 32 μg/ml; 90% inhibited by 0.25 μg/ml), *Haemophilus influenzae* (MICs, 0.03 to 0.12 μg/ml), and *Neisseria gonorrhoeae* (MICs, 0.008 to 0.016 μg/ml). It was also active against *Pseudomonas aeruginosa* (MICs, 0.12 to 2 μg/ml), most other pseudomonads (MICs, 0.03 to 32 μg/ml), and *Acinetobacter calcoaceticus* (MICs, 0.06 to 4 μg/ml). Norfloxacin was somewhat less active against staphylococci (MICs, 0.25 to 4 μg/ml; 1 μg/ml required to inhibit 50% of isolates) and streptococci (MICs, 0.5 to 64 μg/ml). Members of the *Bacteroides fragilis* group of anaerobes were relatively resistant to norfloxacin (MICs, 8 to 128 μg/ml), as were most other anaerobes.

Although resistance to many antimicrobial agents, including gentamicin and many β-lactams, has become fairly common in recent years, resistance to nalidixic acid remains rare. For example, Casewell and Talsania (2) found that only 13% of 108 epidemiologically distinct, gentamicin-resistant klebsiellae were resistant to nalidixic acid; amikacin and β-lactamase-resistant cephalosporins such as cefuroxime, cefotaxime, and cefoxitin were the only compounds that were more frequently effective. The rarity of resistance to nalidixic acid may be because resistance to it is not plasmid mediated (1). Nevertheless, nalidixic acid suffers from its relatively low intrinsic activity and the fact that it is available only for oral administration, which produces low concentrations in the blood. Hence it is used solely for the treatment of urinary tract infections.

Norfloxacin (MK-0366, AM-715) is an analog of nalidixic acid with much greater intrinsic activity (3). In this paper we compare its activity not with nalidixic acid, as the superiority of the activity of norfloxacin over that of nalidixic acid has been shown by Ito et al. (3), but with appropriate β-lactams and, in some cases, gentamicin or metronidazole.

MATERIALS AND METHODS

Organisms. The clinical isolates studied are listed in Tables 1 to 3. Most of the bacteria in the collection, which included many isolates selected for their resistance to β-lactam antibiotics, were from St. Thomas' Hospital, but a few were from other hospitals in England.

Antibiotics. All antibiotics were obtained in England. Norfloxacin was obtained from Merck Sharp & Dohme Research Laboratories, Hoddesdon. Ampicillin, benzylpenicillin (penicillin G), and carbenicillin were provided by Beecham Research Laboratories, Brentford; cephaloridine, by Glaxo Group Research, Greenford; cefotaxime, by Roussel Laboratories, Wembley Park; gentamicin, by Nicholas Laboratories, Slough; and metronidazole, by May and Baker Ltd., Dagenham.

Assessment of antibacterial activity. Minimal inhibitory concentrations (MICs) for enterobacteria were determined by broth dilution in Iso-Sensitest broth (CM 473; Oxoid Ltd., Basingstoke, England) in microtiter trays with the aid of an MIC 2000 dispenser and inoculator (Dynatech Laboratories Ltd., Billingshurst, England). The inoculum size was 10^4 colony-forming units (CFU) per ml (final concentration) unless otherwise stated. Minimal bactericidal concentrations were determined by subculturing 1-μl amounts from the trays in which MICs had been determined into broth (100 μl) without antibiotics and examining for growth after overnight incubation. MICs for other organisms were determined by agar dilution with, unless otherwise stated, an inoculum of 10^4 CFU. The medium used was Diagnostic Sensitivity Test agar (CM 261; Oxoid Ltd.). This was supplemented with 6% saponin-lysed horse blood for fastidious organisms except *Haemophilus influenzae*, for which it was supplemented with 0.25% lysed horse blood and 10 μg of NAD per ml. The compounds with which norfloxacin was compared are shown in Tables 1 to 3.

RESULTS

The activity of norfloxacin against enterobacteria was comparable with that of the newer cephalosporins, such as cefotaxime, and exceeded that of gentamicin, ampicillin, and cephaloridine (Table 1). Over 97% of the isolates were inhibited by 1 μg of the compound per ml; the 10 isolates that were resistant to this concen-

TABLE 1. Activity of norfloxacin against aerobic gram-negative organisms

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)			
		Range	50%	75%	90%
Enterobacteria (397)[a]	Norfloxacin	0.008–32	0.06	0.12	0.25
	Cefotaxime	0.004–128	0.06	0.12	0.25
	Cephaloridine	0.25–>128	32	>128	>128
	Ampicillin	0.25–>128	32	>128	>128
	Gentamicin	0.06–>128	0.5	1	8
A. calcoaceticus (35)[b]	Norfloxacin	0.06–4	1	2	4
	Cefotaxime	0.12–128	1	4	16
	Ampicillin	0.5–>128	4	8	64
	Gentamicin	0.06–128	0.5	1	2
Pseudomonas aeruginosa (50)	Norfloxacin	0.12–2	0.25	1	2
	Cefotaxime	1–128	8	16	64
	Carbenicillin	16–>4096	64	512	4096
	Gentamicin	0.12–128	1	1	2
Pseudomonas spp. (39)[c]	Norfloxacin	0.03–32	1	4	16
	Cefotaxime	0.06–64	4	16	32
	Carbenicillin	2–2048	256	1024	2048
	Gentamicin	0.12–>128	1	32	>128
H. influenzae (27)	Norfloxacin	0.03–0.12	0.03	0.06	0.12
	Ampicillin	0.12–32	0.25	4	16
N. gonorrhoeae (48)	Norfloxacin	0.008–0.016	0.016	0.016	0.016
	Benzylpenicillin	0.008–16	0.06	1	4

[a] *Citrobacter freundii* (20 isolates), *C. koseri* (20 isolates); *Enterobacter aerogenes* (25 isolates), *Enterobacter cloacae* (35 isolates); *Escherichia coli* (50 isolates); *Klebsiella* spp. (50 isolates); *K. ozaenae* (11 isolates); *Morganella morganii* (20 isolates); *Proteus mirabilis* (45 isolates), *Proteus vulgaris* (26 isolates); *Providencia alcalifaciens* (10 isolates), *Providencia rettgeri* (25 isolates), *Providencia stuartii* (35 isolates); and *Serratia* spp. (25 isoaltes).

[b] *A. calcoaceticus* subsp. *anitratus* (20 isolates) and *A. calcoaceticus* subsp. *lwoffi* (15 isolates).

[c] *Pseudomonas acidovorans* (8 isolates), *Pseudomonas cepacia* (6 isolates), *Pseudomonas fluorescens* (5 isolates), *Pseudomonas maltophilia* (7 isolates), *Pseudomonas putida* (10 isolates), *Pseudomonas stutzeri* (2 isolates), and *Pseudomonas thomasii* (1 isolate).

tration comprised 5 isolates of *Providencia stuartii*, 3 of *Providencia rettgeri*, and 1 each of *Enterobacter cloacae* and *Escherichia coli*. All isolates except one of *Providencia rettgeri* (MIC, 32 μg/ml) were inhibited by 4 μg of norfloxacin per ml. Inoculum effects on the MICs of norfloxacin for the 37 enterobacteria tested were small, with the MICs either remaining unchanged or doubling when the inoculum was increased to 10^6 CFU/ml, and increasing two- to fourfold when the inoculum was increased to 10^8 CFU/ml. The minimal bactericidal concentrations were similar to the MICs.

A. calcoaceticus was less susceptible than the enterobacteria to norfloxacin (Table 1). Gentamicin was more active than norfloxacin was against most isolates, but ampicillin, cephaloridine, and cefotaxime were less active.

Norfloxacin was more active than any of the β-lactams and possessed activity comparable to that of gentamicin against *Pseudomonas aeruginosa*, all isolates of which were inhibited by 2 μg of the compound per ml (Table 1). It was also fairly active against most other pseudomonads, with MICs of 4 μg/ml or less, though *Pseudomonas maltophilia* was less susceptible (MICs, 8 to 32 μg/ml).

All of the *H. influenzae* isolates tested, including seven β-lactamase producers, were highly susceptible to norfloxacin, with MICs in the range of 0.03 to 0.12 μg/ml (Table 1). *N. gonorrhoeae* was exquisitely susceptible to the compound, with MICs in the range of 0.008 to 0.016 μg/ml for all of the isolates tested, including 13 β-lactamase producers (Table 1).

Norfloxacin, with MICs in the range of 1 to 4 μg/ml, was less active than gentamicin or cephaloridine against most isolates of *Staphylococcus aureus* (Table 2). There was mostly a two- to fourfold increase in the MICs of norfloxacin when the inoculum was increased to 10^6 CFU. The activity of the compound against the few coagulase-negative staphylococci tested was similar to that against *S. aureus* (Table 2). Nor-

TABLE 2. Activity of norfloxacin against staphylococci and streptococci

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)			
		Range	50%	75%	90%
Staphylococcus aureus (30)[a]	Norfloxacin	0.25–4	1	2	2
	Benzylpenicillin	0.016–16	0.25	8	16
	Cephaloridine	0.016–1	0.12	0.25	0.5
	Cefotaxime	0.5–32	2	8	8
	Gentamicin	0.12–128	0.25	0.5	16
Coagulase-negative staphylococci (9)	Norfloxacin	0.25–2	1	1	2
	Benzylpenicillin	0.016–64	0.25	1	1
	Cephaloridine	0.016–2	0.06	0.12	0.12
	Cefotaxime	1–4	2	4	4
	Gentamicin	0.12–128	0.5	16	32
Beta-hemolytic streptococci, Lancefield groups A, C, and G (40)	Norfloxacin	2–16	4	4	4
	Benzylpenicillin	0.002–0.008	0.004	0.008	0.008
	Cephaloridine	0.002–0.008	0.004	0.008	0.008
	Cefotaxime	0.004–0.016	0.016	0.016	0.016
Streptococcus agalactiae (25)	Norfloxacin	0.5–4	2	2	4
	Benzylpenicillin	0.016–0.03	0.03	0.03	0.03
	Cephaloridine	0.008–0.03	0.016	0.016	0.016
	Cefotaxime	0.016–0.06	0.06	0.06	0.06
Enterococci (20)	Norfloxacin	0.5–4	2	2	4
	Ampicillin	1–32	1	1	1
	Cephaloridine	8–>64	8	16	16
	Cefotaxime	4–>128	>128	>128	>128
Alpha- and nonhemolytic streptococci (37)[c]	Norfloxacin	4–64	8	32	32
	Benzylpenicillin	0.008–0.5	0.03	0.06	0.12
	Cephaloridine	0.002–>1	0.03	0.06	0.12
	Cefotaxime	0.016–>32	0.12	0.25	0.25
Streptococcus pneumoniae (18)	Norfloxacin	4–16	8	8	16
	Benzylpenicillin	0.008–>2	0.016	0.016	0.5
	Cephaloridine	0.016–1	0.03	0.03	0.12
	Cefotaxime	0.004–1	0.016	0.016	0.12

[a] Penicillin susceptible (10 isolates), penicillin resistant (10 isolates), and penicillin and methicillin resistant (10 isolates).

[b] Group A (19 isolates), Group C (3 isolates), and Group G (18 isolates).

[c] *Streptococcus bovis* (6 isolates; 4 type I and 2 type II), *Streptococcus milleri* (9 isolates), *Streptococcus mitior* (3 isolates, including 1 that was dextran-positive), *Streptococcus mutans* (7 isolates), *Streptococcus salivarius* (5 isolates), *Streptococcus sanguis* (6 isolates), and 1 streptococcus that was not identified to species level.

floxacin was considerably less active than benzylpenicillin was against most streptococci (Table 2), although it possessed activity similar to that of ampicillin against enterococci.

Norfloxacin had poor activity against most anaerobes, with considerably lower activity than ampicillin or metronidazole (Table 3). In addition to the results shown in Table 3, its activity was assessed against two isolates of *Eubacterium* spp. (MICs, 4 to 8 μg/ml) and two isolates of *Veillonella* spp. (MIC, 1 μg/ml).

DISCUSSION

Our results confirm the high in vitro activity of norfloxacin against aerobic organisms reported by Ito et al. (3) and against *N. gonorrhoeae* in particular as reported by Khan et al. (4). However, we found the compound to have fairly poor activity against anaerobes, which do not seem to have been studied before.

Urinary levels of norfloxacin are said to exceed 350 μg/ml after an oral dose of 800 mg (*Preclinical Investigator Brochure*, no. MK-0366; Merck Sharp & Dohme Research Laboratories, Rahway, N.J.). Thus, in the context of urinary tract infections, virtually all of the organisms that we tested can be regarded as susceptible to norfloxacin. This large group of susceptible organisms includes multiply resistant gram-negative bacilli and enterococci against

TABLE 3. Activity of norfloxacin against anaerobes

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)			
		Range	50%	75%	90%
B. fragilis group (10)[a]	Norfloxacin	8–128	16	128	128
	Ampicillin	8–32	8	16	16
	Metronidazole	0.25–1	0.5	0.5	0.5
Other *Bacteroides* spp. (17)[b]	Norfloxacin	0.25–128	8	16	64
	Ampicillin	<0.016–>64	4	8	32
	Metronidazole	0.12–32	0.5	2	8
Fusobacterium spp. (6)	Norfloxacin	2–16	8	16	16
	Ampicillin	<0.016–0.25	0.03	0.12	0.25
	Metronidazole	<0.016–0.12	0.03	0.06	0.12
Clostridium spp. (9)	Norfloxacin	2–>128	32	128	>128
	Ampicillin	0.12–4	0.5	2	4
	Metronidazole	0.06–2	0.25	1	2
Anaerobic gram-positive cocci (10)	Norfloxacin	1–128	2	4	16
	Ampicillin	<0.016–1	0.06	0.25	0.5
	Metronidazole	<0.016–2	0.5	1	1

[a] *B. fragilis* (4 isolates), *B. distasonis* (2 isolates), *B. ovatus* (2 isolates), and *B. thetaiotaomicron* (2 isolates).
[b] *B. melaninogenicus* (3 isolates), *B. asaccharolyticus* (1 isolate), *B. oralis* (2 isolates), *B. bivius* (2 isolates), *B. corrodens* (2 isolates), *B. disiens* (2 isolates), *B. ruminicola* (1 isolate), *B. uniformis* (2 isolates), and *Bacteroides* sp. (2 isolates).

which many other new antibiotics have poor activity. The only organisms with doubtful susceptibility to norfloxacin in this context are some bacteroides and clostridia—not common urinary pathogens.

It is less clear whether the compound is likely to have a major role in systemic therapy. Blood levels reach a peak of only about 2.5 μg/ml after an oral dose of 800 mg (*Preclinical Investigator Brochure*, no. MK-0366). Such concentrations may well be sufficient to inhibit all isolates of *H. influenzae*, *N. gonorrhoeae*, and many enterobacteria and pseudomonads, but are not sufficient for the compound to be effective against gram-positive organisms.

From its in vitro activity, norfloxacin appears to be a potentially valuable agent for the treatment of urinary tract infections and gonorrhea. The usefulness of this compound for the treatment of serious infections that require hospitalization has yet to be evaluated, although it may well be useful for the treatment of hospital-acquired urinary tract infections with multiply resistant organisms.

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