ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1983, p. 78–84
0066-4804/83/070078-07$02.00/0

Vol. 24, No. 1

In Vitro and In Vivo Antibacterial Activity of AT-2266

KAORU KOUNO,[1]* MATSUHISA INOUE,[2] AND SUSUMU MITSUHASHI[1]

Department of Microbiology[1] and Laboratory of Drug Resistance in Bacteria,[2] Gunma University School of Medicine, Maebashi, Japan

Received 10 February 1983/Accepted 11 May 1983

AT-2266 [1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid] showed a broad spectrum of antibacterial activity against gram-positive and gram-negative microorganisms, including *Pseudomonas aeruginosa*. The in vitro antibacterial activity of AT-2266 was in general comparable to that of norfloxacin, but much higher than that of pipemidic or nalidixic acid. The 90% minimal inhibitory concentrations (MIC_{90}s) of AT-2266 for *P. aeruginosa* resistant to gentamicin (MIC range, 25 to >200 μg/ml) and *Enterobacteriaceae* resistant to nalidixic acid (25 to >1,600 μg/ml) were 3.13 and 12.5 μg/ml, respectively. The MICs of AT-2266 were only slightly affected by the addition of horse serum or sodium cholate, by the pH of the medium, and by inoculum size. AT-2266 was bactericidal at concentrations near its MIC value. The 50% effective doses of AT-2266 after oral administration against systemic infections in mice were about 1/2 those of norfloxacin, about 1/10 those of pipemidic acid, and between 1/20 and 1/40 those of nalidixic acid.

AT-2266 [1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid] is a new pyridonecarboxylic acid derivative synthesized by Matsumoto et al. (7). This compound, like the structurally related norfloxacin (1–5, 10), has a high order of activity against a broad spectum of gram-positive, gram-negative, and glucose-nonfermenting bacteria. Its activity is in general superior to that of pipemidic acid, nalidixic acid, oxolinic acid, cephalexin, ampicillin, carbenicillin, and gentamicin in vitro and in vivo (11). Oral administration of AT-2266 to animals produced concentrations in body fluids and tissues high enough to cover the minimal inhibitory concentrations (MICs) of a large variety of bacterial pathogens (9). Because there was no evidence of cross-resistance with agents such as streptomycin, chloramphenicol, tetracycline, ampicillin, or cephalexin (11, 12), AT-2266 might be a potentially useful drug against various pathogens which are now resistant to these antibiotics. To estimate the chemotherapeutic value of AT-2266, we compared its in vitro and in vivo antibacterial activities with those of norfloxacin, pipemidic acid, and nalidixic acid.

MATERIALS AND METHODS

Drugs. AT-2266 sesquihydrate and pipemidic acid trihydrate were obtained from Dainippon Pharmaceutical Co., Ltd.; norfloxacin was from Kyorin Pharmaceutical Co., Ltd.; nalidixic acid was from Daiichi Seiyaku Co., Ltd.; and gentamicin was from Shionogi Co., Ltd.

Organisms. The organisms used were reference strains stored in our laboratories and recent clinical isolates randomly collected in various hospitals in Japan.

Determination of MICs. MICs were determined by the twofold agar dilution method. The media used for preculture and MIC determination were brain heart infusion broth and agar (Difco Laboratories), respectively, for streptococci; brain heart infusion broth and agar (Difco) supplemented with hemin (10 μg/ml) and β-NAD (Sigma Chemical Co.) for *Haemophilus influenzae*; GAM broth and agar (Nissui) for obligate anaerobes, and sensitivity test broth and agar (Nissui) for the other microorganisms. A bacterial culture or bacterial suspension in broth was adjusted to the density of a 0.5 McFarland standard (about 10^8 cells per ml). One loopful (5 μl) of the 10^{-2} dilutions of the culture or suspension was inoculated onto 10-ml agar layers containing the drugs at fixed ratios. The plates were incubated at 37°C for 18 h, except for obligate anaerobes, which were incubated for 48 h. *H. influenzae* was cultured in a candle jar, and obligate anaerobes were cultured in an anaerobic glove box. The MIC was defined as the lowest drug concentration which inhibited visible bacterial growth.

Factors affecting MICs. The influence of the inoculum size, the pH of the medium, and the addition of horse serum or sodium cholate on the MIC was tested by the twofold agar dilution method.

Bactericidal activity. Bactericidal activity was tested by the determination of minimal bactericidal concentrations (MBCs) and the reduction of viable cells during exposure to the drug for 24 h. The MBCs were determined as follows. The microorganisms were precultured in trypto-soy broth (Eiken) at 37°C for 18 h and diluted with the same broth to a density of about 10^6 cells per ml. A 1-μl sample of the dilutions was

inoculated into 0.1 ml of broth containing the drug in twofold dilutions, the final bacterial density being about 10^4 cells per ml. The MBC was determined by inoculating 5 μl from clear wells to drug-free sensitivity test agar and was defined as the lowest concentration at which no bacterial growth was visible after 18 h at 37°C. The reduction of viable cells during exposure to the drug was measured as follows. The microorganisms precultured as described above were suspended in 5 ml of sensitivity test broth to a density of about 10^4 cells per ml and incubated for 24 h at 37°C while being shaken. After 2 h, the drug was added to the cultures at a final concentration of approximately the MIC. Samples of 0.1 ml of the cultures were taken periodically, diluted appropriately, and plated on drug-free sensitivity test agar. After incubation at 37°C for 18 h, the CFU were enumerated and regarded as viable cells.

Measurement of the frequency of spontaneous resistant mutants. The microorganisms were precultured in sensitivity test broth at 37°C for 18 h. Volumes of 0.1 ml of the undiluted and the 10-times-diluted cultures were plated on sensitivity test agar containing a drug at a concentration of 8 times the MIC. Three plates were used for each sample. After incubation at 37°C for 48 h, CFU were counted, and the frequency of spontaneous resistant mutants was calculated.

Determination of in vivo activity. The in vivo antibacterial activity of the drugs was determined by measuring their protective effect against systemic infections in mice. Twenty to thirty male STD:ddy mice weighing 19 to 21 g were used for each dose. Each microorganism was cultured in nutrient broth (Eiken) at 37°C for 18 h and diluted appropriately with the same broth containing 4% gastric mucin to a final cell density of 100 times the median lethal dose. A 0.4-ml volume of the bacterial dilution was inoculated intraperitoneally. The inoculum size (cells per mouse) was 1.2×10^5 for *Staphylococcus aureus* Smith, 3.0×10^2 for *Escherichia coli* ML4707, 4.3×10^4 for *Klebsiella pneumoniae* GN6445, and 1.6×10^4 for *Pseudomonas aeruginosa* GN11189. The drugs were suspended in 0.2% carboxymethylcellulose and given as a single oral dose to the mice immediately after infection. Survivors were recorded 1 week postinfection. The 50% effective doses (ED_{50}s) were calculated by the probit method (8), and the 95% confidence limits were calculated by the Litchfield and Wilcoxon method (6).

RESULTS

Susceptibility of clinical isolates. The susceptibility of recent clinical isolates to AT-2266, norfloxacin, pipemidic acid, and nalidixic acid is shown in Table 1. The MIC_{90}s (the concentration at which more than 90% of the isolates were inhibited) of AT-2266 ranged from 0.2 to 1.56 μg/ml for *S. aureus, Staphylococcus epidermidis*, and most gram-negative organisms, including *P. aeruginosa* and *H. influenzae*. For *Streptococcus pyogenes*, some gram-negative organisms such as *Citrobacter freundii* and *Serratia marcescens*, the glucose-nonfermenting organisms such as *Pseudomonas cepacia, Pseudomonas maltophilia*, and *Acinetobacter calcoaceticus*, the obligate anaerobes such as *Bacteroides fragilis, Clostridium perfringens*, and *Clostridium difficile*, the nalidixic acid-resistant strains of *Enterobacteriaceae*, and the gentamicin-resistant strains of *P. aeruginosa*, the MIC_{90}s ranged from 3.13 to 25 μg/ml.

The MIC_{90}s of AT-2266 were equal to or two to four times lower than those of norfloxacin for gram-positive microorganisms, the glucose-nonfermenting strains, and the obligate anaerobes, except for *C. perfringens*, but they were equal to or two times higher than those of norfloxacin for the gram-negative strains. The MIC_{90}s of pipemidic acid and nalidixic acid were in general much higher than those of AT-2266 and norfloxacin. AT-2266 was also active against gentamicin-resistant strains of *P. aeruginosa*. *Enterobacteriaceae* isolates highly resistant to nalidixic acid were moderately resistant to AT-2266 and norfloxacin, but 90% were still inhibited at a concentration of 12.5 μg/ml.

The MICs of AT-2266 were only slightly affected by the addition of horse serum or sodium cholate, pH of the medium, and inoculum size (data not shown).

Bactericidal activity. The bactericidal activity of AT-2266 compared with that of norfloxacin, pipemidic acid, and nalidixic acid is shown in Table 2. The 90% minimal bactericidal concentrations (MBC_{90}s) of AT-2266 were equal to or two times higher than the MIC_{90}s against 20 recent clinical isolates of *S. aureus, E. coli, K. pneumoniae*, and *P. aeruginosa*. The analogous compounds used for reference showed a similar ratio between MIC_{90} and MBC_{90} values. AT-2266 was also bactericidal at concentrations twice the MIC for selected strains of *S. aureus, E. coli, K. pneumoniae*, and *P. aeruginosa* (Fig. 1). No regrowth of the test organisms was observed throughout 24 h of incubation.

Frequency of spontaneous resistant mutants. Table 3 shows the frequencies of spontaneous mutants resistant to AT-2266, norfloxacin, pipemidic acid, and nalidixic acid. Mutants resistant to nalidixic acid were found in 8 of the 10 microorganisms tested. In two cases, spontaneous pipemidic acid-resistant strains were isolated. No mutants resistant to AT-2266 or norfloxacin were found at a detectable frequency.

In vivo antibacterial activity. The protective effects of single oral doses of AT-2266, norfloxacin, pipemidic acid, and nalidixic acid against systemic infections with *S. aureus* Smith, *E. coli* ML4707, *K. pneumoniae* GN6445, and *P. aeruginosa* GN11189 in mice are shown in Table 4. The ED_{50}s of AT-2266 were about 1/2 those of norfloxacin, 1/9 to 1/11 those of pipemidic acid, and 1/24 to 1/42 those of nalidixic acid.

It was remarkable that AT-2266 was in general more active than norfloxacin in vivo, irrespec-

TABLE 1. Susceptibility of fresh clinical isolates to AT-2266, norfloxacin, pipemidic acid, and nalidixic acid

Organism (no. of strains)	Drug	MIC (μg/ml)[a]		
		Range	50%	90%
S. aureus (104)	AT-2266	0.39–3.13	1.56	1.56
	Norfloxacin	0.39–12.5	1.56	3.13
	Pipemidic acid	6.25–100	50	50
	Nalidixic acid	12.5–100	25	50
S. epidermidis (54)	AT-2266	0.39–1.56	0.78	0.78
	Norfloxacin	0.39–1.56	0.78	1.56
	Pipemidic acid	12.5–100	50	50
	Nalidixic acid	12.5–50	50	50
S. pyogenes (101)	AT-2266	1.56–50	3.13	12.5
	Norfloxacin	0.78–50	1.56	12.5
	Pipemidic acid	100–>100	>100	>100
	Nalidixic acid	>100	>100	>100
E. coli (98)	AT-2266	0.05–3.13	0.2	0.78
	Norfloxacin	0.025–1.56	0.2	0.39
	Pipemidic acid	0.78–25	1.56	3.13
	Nalidixic acid	0.78–>100	3.13	6.25
K. pneumoniae (44)	AT-2266	0.1–3.13	0.39	0.39
	Norfloxacin	0.1–1.56	0.2	0.39
	Pipemidic acid	0.78–25	3.13	6.25
	Nalidixic acid	0.78–>100	3.13	6.25
Klebsiella oxytoca (41)	AT-2266	0.1–1.56	0.2	0.78
	Norfloxacin	0.1–0.78	0.2	0.39
	Pipemidic acid	0.78–12.5	1.56	6.25
	Nalidixic acid	1.56–>100	3.13	6.25
C. freundii (51)	AT-2266	0.1–12.5	0.39	6.25
	Norfloxacin	0.1–12.5	0.2	6.25
	Pipemidic acid	0.78–100	3.13	50
	Nalidixic acid	1.56–>100	6.25	>100
Enterobacter cloacae (99)	AT-2266	0.1–12.5	0.39	1.56
	Norfloxacin	0.05–6.25	0.2	0.78
	Pipemidic acid	0.78–100	1.56	12.5
	Nalidixic acid	1.56–>100	3.13	100
Proteus mirabilis (100)	AT-2266	0.1–1.56	0.39	0.39
	Norfloxacin	0.05–0.78	0.2	0.2
	Pipemidic acid	0.78–12.5	3.13	3.13
	Nalidixic acid	1.56–>100	3.13	6.25
Indole-positive *Proteus* spp. (205)[b]	AT-2266	0.05–6.25	0.39	0.78
	Norfloxacin	0.025–6.25	0.1	1.56
	Pipemidic acid	0.78–100	1.56	6.25
	Nalidixic acid	0.78–>100	1.56	25
Salmonella spp. (100)	AT-2266	0.1–0.78	0.39	0.39
	Norfloxacin	0.05–0.39	0.2	0.2
	Pipemidic acid	0.78–6.25	1.56	1.56
	Nalidixic acid	1.56–12.5	3.13	6.25
Shigella spp. (105)	AT-2266	0.05–1.56	0.1	0.2
	Norfloxacin	0.025–0.39	0.1	0.1
	Pipemidic acid	0.39–12.5	0.78	1.56
	Nalidixic acid	0.39–100	1.56	1.56

TABLE 1—*Continued*

Organism (no. of strains)	Drug	MIC (μg/ml)[a]		
		Range	50%	90%
S. marcescens (105)	AT-2266	0.2–50	0.78	25
	Norfloxacin	0.1–50	0.78	25
	Pipemidic acid	1.56–>100	6.25	>100
	Nalidixic acid	0.78–>100	3.13	>100
Nalidixic acid-resistant *Enterobacteriaceae* (62)[c]	AT-2266	0.39–100	3.13	12.5
	Norfloxacin	0.2–100	1.56	12.5
	Pipemidic acid	6.25–400	25	100
	Nalidixic acid	25–>1600	400	>1,600
P. aeruginosa (97)	AT-2266	0.39–6.25	0.78	1.56
	Norfloxacin	0.39–6.25	0.78	1.56
	Pipemidic acid	6.25–50	12.5	25
	Nalidixic acid	25–>100	50	100
	Gentamicin	0.2–>100	1.56	6.25
Gentamicin-resistant *P. aeruginosa* (56)	AT-2266	0.39–25	1.56	3.13
	Gentamicin	25–>200	200	>200
P. cepacia (51)	AT-2266	3.13–50	12.5	25
	Norfloxacin	6.25–50	25	25
	Pipemidic acid	50–>100	100	>100
	Nalidixic acid	12.5–100	25	50
P. maltophilia (52)	AT-2266	1.56–50	12.5	25
	Norfloxacin	3.13–100	25	100
	Pipemidic acid	25–>100	100	>100
	Nalidixic acid	1.56–100	12.5	25
A. calcoaceticus (39)	AT-2266	0.39–25	1.56	12.5
	Norfloxacin	0.78–25	3.13	25
	Pipemidic acid	6.25–>100	50	>100
	Nalidixic acid	1.56–50	3.13	12.5
H. influenzae (41)	AT-2266	0.05–0.2	0.2	0.2
	Norfloxacin	0.025–0.2	0.05	0.1
	Pipemidic acid	1.56–6.25	3.13	3.13
	Nalidixic acid	0.78–3.13	1.56	3.13
B. fragilis (34)	AT-2266	6.25–50	12.5	25
	Norfloxacin	25–>100	50	100
	Pipemidic acid	50–>100	100	>100
	Nalidixic acid	100–>100	100	>100
C. perfringens (27)	AT-2266	1.56–3.13	1.56	3.13
	Norfloxacin	0.78–1.56	1.56	1.56
	Pipemidic acid	12.5–50	25	50
	Nalidixic acid	6.25–50	25	50
C. difficile (17)	AT-2266	12.5–25	25	25
	Norfloxacin	25–50	50	50
	Pipemidic acid	50–100	100	100
	Nalidixic acid	>100	>100	>100

[a] Determined by the agar dilution method. 50% and 90%, MIC inhibiting 50 and 90% of strains, respectively.
[b] This group included 53 *Proteus morganii* strains, 49 *Proteus vulgaris* strains, 49 *Proteus rettgeri* strains, and 54 *Proteus inconstans* strains.
[c] This group included 9 *E. coli* strains, 6 *K. pneumoniae* strains, 2 *Shigella flexneri* strains, 2 *Shigella sonnei* strains, 2 *P. mirabilis* strains, 1 *P. vulgaris* strain, 3 *P. morganii* strains, 7 *P. rettgeri* strains, 11 *E. cloacae* strains, and 19 *S. marcescens* strains.

TABLE 2. Bactericidal activities of AT-2266, norfloxacin, pipemidic acid, and nalidixic acid against fresh clinical isolates

Organism (no. of strains)	Drug	MIC (μg/ml)[a]		MBC (μg/ml)[b]	
		Range	90%	Range	90%
S. aureus (20)	AT-2266	0.39–3.13	1.56	0.78–3.13	1.56
	Norfloxacin	0.78–3.13	1.56	0.78–3.13	3.13
	Pipemidic acid	25–100	50	25–100	100
	Nalidixic acid	25–50	50	25–100	100
E. coli (20)	AT-2266	0.05–0.39	0.2	0.05–0.39	0.2
	Norfloxacin	0.025–0.2	0.1	0.025–0.39	0.1
	Pipemidic acid	0.78–1.56	1.56	0.78–3.13	3.13
	Nalidixic acid	0.39–6.25	3.13	0.78–6.25	3.13
K. pneumoniae (20)	AT-2266	0.05–0.39	0.2	0.05–0.39	0.2
	Norfloxacin	0.025–0.1	0.05	0.05–0.2	0.1
	Pipemidic acid	0.78–3.13	1.56	0.78–6.25	6.25
	Nalidixic acid	1.56–25	12.5	3.13–50	12.5
P. aeruginosa (20)	AT-2266	0.2–1.56	0.78	0.2–3.13	1.56
	Norfloxacin	0.2–1.56	0.78	0.39–3.13	1.56
	Pipemidic acid	3.13–25	12.5	6.25–50	12.5
	Nalidixic acid	50–>100	>100	50–>100	>100

[a] Determined by the broth dilution method. 90%, MIC inhibiting 90% of strains.
[b] 90%, MBC killing 90% of strains.

tive of its lower in vitro activity against the microorganisms used.

DISCUSSION

AT-2266 showed a broad antibacterial spectrum covering gram-positive and gram-negative microorganisms. Its in vitro antibacterial activity was comparable to that of norfloxacin but higher than that of pipemidic acid or nalidixic acid. The MICs of AT-2266 were only slightly affected by the addition of horse serum or sodium cholate, pH of the medium, and inoculum size. AT-2266, like other pyridone-derived car-

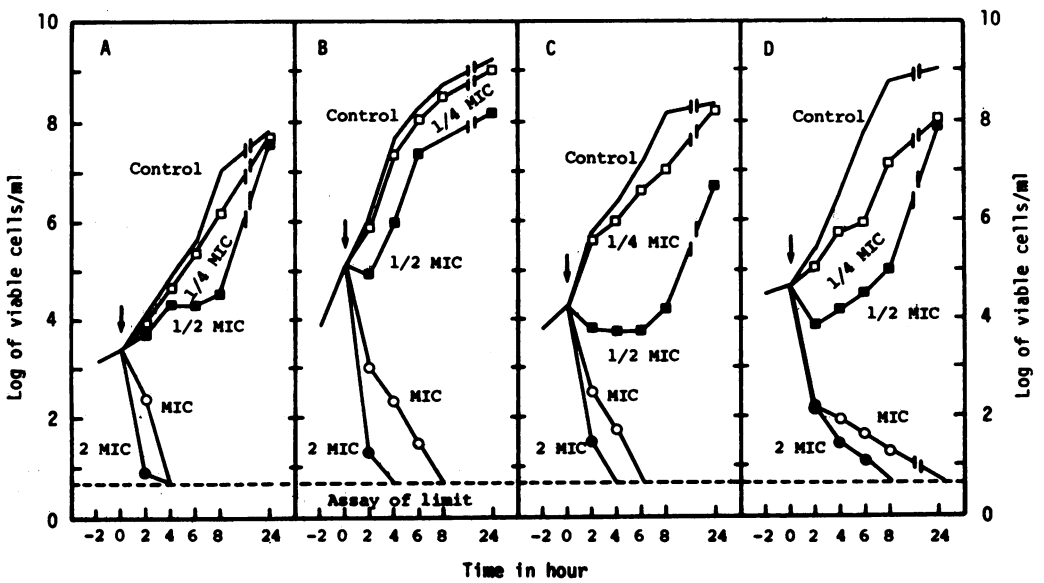

FIG. 1. Bactericidal activity of AT-2266. (A) *S. aureus* Smith (MIC, 0.78 μg/ml); (B) *E. coli* ML4707 (MIC, 0.1 μg/ml); (C) *K. pneumoniae* GN6445 (MIC, 0.2 μg/ml); (D) *P. aeruginosa* GN11189 (MIC, 1.56 μg/ml). The number of viable cells at the appropriate time intervals after addition of AT-2266 (arrow) was counted on drug-free agar.

TABLE 3. Frequency of spontaneous mutants resistant to AT-2266, norfloxacin, pipemidic acid, and nalidixic acid[a]

Organism	Frequency of spontaneous mutants resistant to:			
	AT-2266	Norfloxacin	Pipemidic acid	Nalidixic acid
S. aureus FDA209P JC-1	$<6.8 \times 10^{-9}$	$<6.8 \times 10^{-9}$	2.7×10^{-6}	9.9×10^{-6}
E. coli NIJH JC-2	$<7.1 \times 10^{-9}$	$<7.1 \times 10^{-9}$	$<7.1 \times 10^{-9}$	4.3×10^{-8}
K. pneumoniae PCI-206	$<3.0 \times 10^{-8}$	$<3.0 \times 10^{-8}$	$<3.0 \times 10^{-8}$	2.8×10^{-6}
Salmonella typhimurium IID971	$<8.7 \times 10^{-9}$	$<8.7 \times 10^{-9}$	$<8.7 \times 10^{-9}$	2.3×10^{-8}
P. mirabilis IFO3949	$<5.6 \times 10^{-9}$	$<5.6 \times 10^{-9}$	$<5.6 \times 10^{-9}$	$<5.6 \times 10^{-9}$
P. vulgaris OX19	$<9.7 \times 10^{-9}$	$<9.7 \times 10^{-9}$	$<9.7 \times 10^{-9}$	4.4×10^{-8}
P. morganii IFO3848	$<6.7 \times 10^{-9}$	$<6.7 \times 10^{-9}$	$<6.7 \times 10^{-9}$	$<6.7 \times 10^{-9}$
E. cloacae 963	$<4.1 \times 10^{-9}$	$<4.1 \times 10^{-9}$	$<4.1 \times 10^{-9}$	1.3×10^{-8}
S. marcescens IAM184	$<5.0 \times 10^{-9}$	$<5.0 \times 10^{-9}$	$<5.0 \times 10^{-9}$	6.9×10^{-8}
P. aeruginosa NCTC10490	$<1.6 \times 10^{-9}$	$<1.6 \times 10^{-9}$	7.8×10^{-8}	2.9×10^{-7}

[a] The selective concentration of drug was eight times the MIC.

TABLE 4. In vivo antibacterial activities of AT-2266, norfloxacin, pipemidic acid, and nalidixic acid on systemic infections in mice

Organism	Drug[a]	MIC (μg/ml)	ED_{50} (mg/kg per dose)	95% Confidence limit
S. aureus Smith	AT-2266	0.78	10.3	8.14–12.8
	Norfloxacin	0.39	16.4	12.8–20.9
	Pipemidic acid	6.25	94.4	70.5–126
	Nalidixic acid	12.5	443	308–638
E. coli ML4707	AT-2266	0.1	1.73	1.30–2.30
	Norfloxacin	0.05	2.92	1.97–4.31
	Pipemidic acid	0.78	15.1	11.8–19.2
	Nalidixic acid	1.56	40.8	34.0–49.0
K. pneumoniae GN6445	AT-2266	0.2	0.983	0.592–1.63
	Norfloxacin	0.1	1.86	1.18–2.94
	Pipemidic acid	0.78	8.53	6.85–10.5
	Nalidixic acid	1.56	34.8	25.6–47.5
P. aeruginosa GN11189	AT-2266	1.56	12.2	8.24–17.9
	Norfloxacin	0.78	24.8	17.4–35.5
	Pipemidic acid	12.5	130	96.5–175
	Nalidixic acid	100	308	235–402

[a] Single oral administration immediately after infection.

boxylates, was bactericidal. In general, the antibacterial properties of AT-2266 were very similar to those of norfloxacin (3, 9). However, it was interesting that irrespective of the in vitro susceptibility of the microorganisms to AT-2266 or norfloxacin, AT-2266 showed a higher in vivo activity (Table 4). This might reflect better oral absorption and broader tissue distribution (9). The data in this and previous studies (9, 11) suggest that AT-2266 would be useful in the treatment of systemic infections in addition to localized ones where structurally related compounds have been applied. The toxicity of AT-2266 appears to be rather low (9), a finding that, with others, favors clinical testing of the agent.

ACKNOWLEDGMENTS

We thank S. Nakamura and E. Stobberingh for their advice and criticism during the preparation of this manuscript.

LITERATURE CITED

1. **Downs, J., V. T. Andriole, and J. L. Ryan.** 1982. In vitro activity of MK-0366 against clinical urinary pathogens including gentamicin-resistant *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. 21:670–672.
2. **Hirai, K., A. Ito, Y. Abe, S. Suzue, T. Irikura, M. Inoue, and S. Mitsuhashi.** 1981. Comparative activities of AM-715 and pipemidic and nalidixic acids against experimentally induced systemic and urinary tract infections. Antimicrob. Agents Chemother. 19:188–189.
3. **Ito, A., K. Hirai, M. Inoue, H. Koga, S. Suzue, T. Irikura, and S. Mitsuhashi.** 1980. In vitro antibacterial activity of AM-715, a new nalidixic acid analog. Antimicrob. Agents Chemother. 17:103–108.
4. **Khan, M. Y., R. P. Gruninger, S. M. Nelson, and R. E. Klicker.** 1982. Comparative in vitro activity of norfloxacin (MK-0366) and ten other oral antimicrobial agents against urinary bacterial isolates. Antimicrob. Agents Chemother. 21:848–851.
5. **King, A., C. Warren, K. Shannon, and I. Phillips.** 1982. In vitro antibacterial activity of norfloxacin (MK-0366). Antimicrob. Agents Chemother. 21:604–607.

6. **Litchfield, J. T., Jr., and F. Wilcoxon.** 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. **96**:99–113.
7. **Matsumoto, J., T. Miyamoto, A. Minamida, Y. Nishimura, H. Egawa, and H. Nishimura.** 1980. Structure-activity relationships of 4-oxo-1,8-naphthyridine-3-carboxylic acids including AT-2266, a new oral antipseudomonal agent, p. 454–456. *In* J. D. Nelson and C. Grassi (ed.), Current chemotherapy and infectious disease. American Society for Microbiology, Washington, D.C.
8. **Miller, L. C., and M. L. Tainter.** 1944. Estimation of the ED_{50} and its error by means of logarithmic-probit graph paper. Proc. Soc. Exp. Biol. Med. **57**:261–264.
9. **Nakamura, S., Y. Takase, N. Kurobe, S. Kashimoto, and M. Shimizu.** 1980. Pharmacological properties of AT-2266, p. 456–458. *In* J. D. Nelson and C. Grassi (ed.), Current chemotherapy and infectious disease. American Society for Microbiology, Washington, D.C.
10. **Neu, H. C., and P. Labthavikul.** 1982. In vitro activity of norfloxacin, a quinolinecarboxylic acid, compared with that of β-lactams, aminoglycosides, and trimethoprim. Antimicrob. Agents Chemother. **22**:23–27.
11. **Shimizu, M., Y. Takase, S. Nakamura, H. Katae, S. Inoue, A. Minami, K. Nakata, and Y. Sakaguchi.** 1980. AT-2266, a new oral antipseudomonal agent, p. 451–454. *In* J. D. Nelson and C. Grassi (ed.), Current chemotherapy and infectious disease. American Society for Microbiology, Washington, D.C.
12. **Shimizu, M., Y. Takase, S. Nakamura, H. Katae, A. Minami, K. Nakata, S. Inoue, M. Ishiyama, and Y. Kubo.** 1975. Pipemidic acid, a new antibacterial agent active against *Pseudomonas aeruginosa*: in vitro properties. Antimicrob. Agents Chemother. **8**:132–138.