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In Vitro and In Vivo Antibacterial Activities of E-4868, a New Fluoroquinolone with a 7-Azetidin Ring Substituent

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E-4868, (−)-7-[3-(*R*)-amino-2-(*S*)-methyl-1-azetidinyl]-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxo-3-quinolinecarboxylic acid, is a new fluoroquinolone with an azetidine moiety at the 7 position. The in vitro activity of E-4868 has been compared with those of ciprofloxacin, ofloxacin, and fleroxacin, while the activity of ciprofloxacin was used as reference for in vivo studies. The MICs of E-4868 for 90% of the isolates tested (MIC_{90}s) were 0.06 to 0.5 μg/ml against gram-positive organisms, including *Staphylococcus*, *Streptococcus*, and *Enterococcus* spp. In general, the in vitro potency of E-4868 against gram-positive bacteria was higher than those of all of the other fluoroquinolones tested. MIC_{90}s against members of the family *Enterobacteriaceae* between 0.03 and 1 μg/ml were observed, with the exception of those against *Serratia marcescens* and *Providencia* spp., and a MIC_{90} of 2 μg/ml against *Pseudomonas aeruginosa* was obtained. E-4868 inhibited 90% of the *Clostridium* spp. and *Bacteroides* spp. at 2 μg/ml and was twofold more active than ciprofloxacin. An increase in the Mg^{2+} concentration from 1 to 10 mM increased the MIC between two and three times. Human urine caused a significant decrease in activity of E-4868, which was more pronounced at pH 5.5 than at pH 7.2. The presence of serum also decreased the activity of E-4868. Fifty percent effective dose (ED_{50}) values against experimental *Escherichia coli* HM-42 infections in mice were 3.9 mg/kg of body weight with E-4868 and 3.5 mg/kg of body weight with ciprofloxacin. Corresponding ED_{50} values against *P. aeruginosa* HS-116 were 93.2 and 107.8 mg/kg, respectively, and those against *Staphylococcus aureus* HS-93 were 6.5 and 44.6 mg/kg, respectively. In experimental infections with *Streptococcus pneumoniae* 84551, the ED_{50} value of E-4868 was 154.4 mg/kg, while ciprofloxacin proved totally inactive at a dose of 400 mg/kg. When E-4868 was administered orally at a dose of 50 mg/kg in mice, the area under the concentration-time curve (0 to 4 h) value was 28.4 μg · h/ml, while an area under the concentration-time curve value of 2.3 μg · h/ml was observed for ciprofloxacin at the same dose. In these studies, levels of the two agents in blood 1 h postadministration were 7.6 and 1.2 μg/ml, respectively.

In last few years many new pyridobenzoxazine, naphthyridine, or quinolone derivatives, including ofloxacin (20), enoxacin (14), tosufloxacin (5), norfloxacin (13), pefloxacin (23), ciprofloxacin (25, 30), fleroxacin (1), and others (3, 4, 6–8, 10, 11, 17, 21, 24, 26, 27), have been developed in order to obtain improved activity and wider antibacterial spectra than those of earlier nalidixic and oxolinic acid-type antibacterial agents (2, 28). Most of the newer fluoroquinolones have been used extensively because of their potent activity against gram-negative bacteria (including members of the family *Enterobacteriaceae*, nonfermentative bacilli, *Neisseria* spp., and *Haemophilus* spp.) (27), their good tissue penetration, and their low incidence of adverse effects (12). However, most have little activity against gram-positive bacteria such as staphylococci and streptococci and against anaerobes.

E-4868, (−)-7-[3-(*R*)-amino-2-(*S*)-methyl-1-azetidinyl]-1-(2,4-difluorophenyl)-1,4-dihydro-6-fluoro-4-oxo-3-quinolinecarboxylic acid (Fig. 1), is a new fluoroquinolone antibacterial agent with a new heterocycle at the 7 position of the quinolone ring that has improved activity against several gram-positive organisms. We compared the in vitro activity of E-4868 with those of ciprofloxacin, fleroxacin, and ofloxacin against several groups of clinical isolates. The effects of various assay conditions, such as high Mg^{2+} levels, urine, and serum, on the in vitro activity of E-4868 were determined. We also compared the in vivo protective effect of E-4868 with that of ciprofloxacin against lethal systemic infections in mice.

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MATERIALS AND METHODS

Antibacterial agents. E-4868 and ofloxacin were synthesized at Laboratorios Esteve S.A. (Barcelona, Spain). Ciprofloxacin and fleroxacin were provided by Bayer A.G. (Wuppertal, Germany) and Roche S.A. (Madrid, Spain), respectively. For determination of MICs, a stock solution of 1.6 mg/ml was prepared in 0.1 N NaOH and diluted in broth medium. For in vivo tests, antibacterial agents were dissolved in 0.1 N NaOH, appropriately diluted with sterile water, and finally mixed in 0.1% carboxymethyl cellulose.

Organisms. The organisms used in this study were clinical isolates randomly obtained from various hospitals in Spain (7–9). All strains were stored frozen at −70°C before use.

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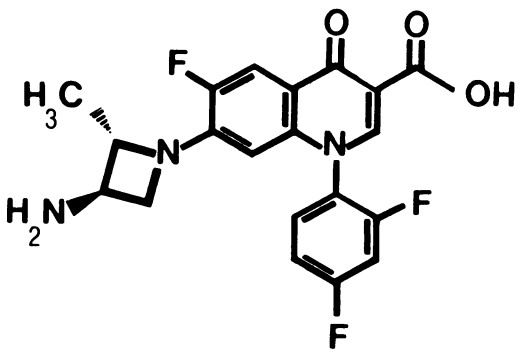

FIG. 1. Chemical structure of E-4868.

Determination of MICs and MBCs. For aerobic and facultatively anaerobic organisms, MICs were determined with liquid medium by the twofold serial antibiotic dilution technique (18). A Quick Spense II microdilution system (Dynatech, Chantilly, Va.) was used to prepare broth microdilution panels containing twofold dilutions of antibacterial agent in 0.15 ml of Mueller-Hinton (MH) broth (Oxoid Ltd., Basingstoke, England). For *Streptococcus* spp., brain heart infusion broth (Oxoid Ltd.) was used. Panels were inoculated with each test organism to yield a final inoculum of 6×10^4 CFU/ml. The MIC in liquid medium was defined as the lowest concentration of antibacterial agent that inhibited development of visible growth after 18 h of incubation at 37°C. MBCs, defined as the lowest antibiotic concentration that killed ≥99.9% of the initial inoculum, were determined by subculturing 10 μl of broth from the drug-free control well, the first well containing growth, and each clear well on MH agar plates (Oxoid Ltd.).

Anaerobic bacteria were tested by the agar dilution method on Wilkins-Chalgren agar medium (Oxoid Ltd.) at 35°C for 48 h in anaerobic GasPak jars (BBL Microbiology Systems, Cockeysville, Md.) (19). The plates were inoculated with a Steers-type multipoint inoculator (22), which deposited approximately 10^4 CFU on the agar surface. Two plates of test medium without an antibacterial agent were also incubated. One was incubated anaerobically to serve as a growth control, and the other was incubated aerobically to detect possible aerobic contamination. The MIC on solid medium was defined as the lowest antibacterial agent concentration that inhibited development of visible growth on agar.

Staphylococcus aureus ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Clostridium perfringens* ATCC 13124, and *Bacteroides fragilis* ATCC 25285, obtained from the American Type Culture Collection (Rockville, Md.), were used as susceptibility test controls.

Factors affecting in vitro activity. The effects of serum, urine, and increasing magnesium concentration on the in vitro activity of E-4868 against *S. aureus* HS-93, *E. coli* HM-42, *Salmonella enteritidis* HSP-928/F, *Klebsiella pneumoniae* HSP-30, *Citrobacter freundii* HSP-73, *Enterobacter aerogenes* HSP-145, *Proteus vulgaris* HSP-99, and *P. aeruginosa* HSP-116 were determined as described above for aerobic and facultatively anaerobic organisms.

(i) Mg^{2+} concentration. The effect of magnesium was determined with unsupplemented MH broth and MH broth supplemented with 1, 5, and 10 mM Mg^{2+} as $MgCl_2 \cdot 6H_2O$.

(ii) Serum. The effect of serum was studied with MH broth supplemented with horse serum (Oxoid Ltd.) (inactivated at 56°C for 30 min) to a final concentration of 20 or 70% (vol/vol) with the pH adjusted to 7.2. Serum-supplemented wells without antibacterial agents were used as controls.

(iii) Urine. The effect of urine on E-4868 activity was determined with early-morning pooled urine samples obtained from healthy human male volunteers. The urine was adjusted to pH 5.5 or 7.2 and subsequently sterilized by passage through a 0.22-μm (pore size) membrane filter (Millipore Corp., Bedford, Mass.).

Mouse protection tests. The following organisms were used in mouse protection tests: *S. aureus* HS-93, *E. coli* HM-42, *P. aeruginosa* HS-116, and *S. pneumoniae* 84551. The MICs and MBCs for these organisms were determined beforehand, as described above.

The organisms were cultured for 12 h at 37°C on MH agar plates (Oxoid Ltd.), except *S. pneumoniae* 84551, which was grown on MH agar supplemented with 5% horse blood and incubated for 12 h at 37°C in a candle jar. The resulting growth was collected from the plates with sterile physiological saline solution. The procedures for infection and treatment were as follows: male HC:CFLP mice weighing approximately 25 g were inoculated intraperitoneally with 0.5 ml of a bacterial suspension adjusted with physiological saline solution to yield five times the minimal lethal dose. The challenge inoculum was sufficient to kill 100% of the untreated control mice, which died within 48 h postinfection, with the exception of mice infected with *S. aureus* HS-93 and *S. pneumoniae* 84551, which died within 4 days after challenge. Immediately after challenge, the mice received a single oral administration of test compound, with the exception of those inoculated with *S. pneumoniae* 84551, which were treated with additional doses at 6, 12, and 24 h after infection. Four groups of 10 mice each were treated with different doses of each antibacterial agent.

The 50% effective dose (ED_{50}) and 95% confidence limits were calculated by probit analysis (16) and by the method of Litchfield and Wilcoxon (15), respectively, 7 days after infection.

Pharmacokinetic study. Male Swiss mice (Charles River) weighing approximately 30 g were used after an acclimatization period on standard diet. Drugs were administered orally at 50 mg/kg of body weight after a 4-h fast. Blood was sampled at 30, 60, 120, and 240 min postadministration, and six mice were killed at each period. Plasma was immediately separated and stored at −20°C until analysis.

The concentration of antibiotic in plasma was measured by agar diffusion under standard conditions with Oxford cylinders 7 mm in diameter. *Bacillus subtilis* ATCC 6633 was used as the indicator organism. The lower limit of detection was <0.05 μg/ml. The areas under the concentration-time curve (AUCs) from 0 to 4 h were calculated from the mean concentrations of the various substances as a function of time by the trapezoidal method.

RESULTS

In vitro activity. MICs of E-4868, fleroxacin, ofloxacin, and ciprofloxacin against several groups of clinical isolates are shown in Table 1. Against some gram-positive organisms, E-4868 showed greater activity than the reference quinolones. Only in the case of methicillin-resistant *Staphylococcus* spp. were MICs for 90% of the strains tested (MIC_{90}s) equal to those of ciprofloxacin and ofloxacin, and for *Enterococcus faecalis*, MICs were equal to those of ciprofloxacin. For the other gram-positive microorganisms,

TABLE 1. In vitro activities of E-4868 and other reference quinolones

Organism (no. tested)	Antibacterial agent	MIC (μg/ml)		
		Range	50%	90%
S. aureus (26)	E-4868	0.015–0.25	0.03	0.06
	Ciprofloxacin	0.03–0.25	0.06	0.25
	Ofloxacin	0.12–1	0.25	0.5
	Fleroxacin	0.12–2	0.25	0.5
Staphylococcus spp.				
Coagulase negative (19)	E-4868	0.03–0.12	0.06	0.12
	Ciprofloxacin	0.06–0.25	0.12	0.25
	Ofloxacin	0.12–1	0.25	0.5
	Fleroxacin	0.12–1	0.5	0.5
Methicillin susceptible (24)	E-4868	0.03–0.25	0.06	0.12
	Ciprofloxacin	0.06–2	0.25	0.5
	Ofloxacin	0.25–1	0.25	0.5
	Fleroxacin	0.25–4	0.25	1
Methicillin resistant (13)	E-4868	0.06–0.5	0.12	0.5
	Ciprofloxacin	0.12–2	0.25	0.5
	Ofloxacin	0.12–1	0.25	0.5
	Fleroxacin	0.12–4	0.5	1
E. faecalis (29)	E-4868	0.12–1	0.25	0.5
	Ciprofloxacin	0.25–1	0.25	0.5
	Ofloxacin	0.5–2	1	2
	Fleroxacin	1–4	2	4
S. pneumoniae (10)	E-4868	0.03–0.12	0.06	0.12
	Ciprofloxacin	0.12–1	0.25	1
	Ofloxacin	0.12–1	0.5	1
	Fleroxacin	1–4	2	4
Viridans group streptococci (8)	E-4868	0.12–0.25	0.12	
	Ciprofloxacin	0.25–2	1	
	Ofloxacin	0.25–2	1	
	Fleroxacin	2–8	4	
E. coli (43)	E-4868	0.015–2	0.03	0.06
	Ciprofloxacin	≤0.007–1	0.007	0.015
	Ofloxacin	0.03–2	0.06	0.12
	Fleroxacin	0.03–2	0.06	0.12
E. cloacae (10)	E-4868	0.03–0.5	0.06	0.25
	Ciprofloxacin	≤0.007–0.5	0.015	0.03
	Ofloxacin	0.06–1	0.12	0.25
	Fleroxacin	0.06–2	0.12	0.25
E. agglomerans (9)	E-4868	0.015–0.12	0.06	
	Ciprofloxacin	≤0.007–0.03	0.015	
	Ofloxacin	0.03–0.12	0.12	
	Fleroxacin	0.06–0.12	0.12	
S. marcescens (20)	E-4868	0.06–8	0.5	4
	Ciprofloxacin	0.015–1	0.06	1
	Ofloxacin	0.06–4	0.25	2
	Fleroxacin	0.06–4	0.25	1
C. freundii (16)	E-4868	0.06–0.25	0.06	0.25
	Ciprofloxacin	≤0.007–0.12	0.015	0.06
	Ofloxacin	0.06–0.25	0.12	0.25
	Fleroxacin	0.06–0.5	0.12	0.5
K. pneumoniae (19)	E-4868	0.03–1	0.12	0.5
	Ciprofloxacin	0.03–0.5	0.06	0.5
	Ofloxacin	0.12–1	0.12	1
	Fleroxacin	0.12–1	0.25	1
K. oxytoca (7)	E-4868	0.03–0.06	0.06	
	Ciprofloxacin	≤0.007–0.03	0.015	
	Ofloxacin	0.06–0.12	0.06	
	Fleroxacin	0.06–0.12	0.06	

Continued on following page

TABLE 1—*Continued*

Organism (no. tested)	Antibacterial agent	MIC (μg/ml)		
		Range	50%	90%
S. typhi (16)	E-4868	≤0.007–0.06	0.015	0.03
	Ciprofloxacin	≤0.007–0.015	0.015	0.015
	Ofloxacin	0.03–0.06	0.06	0.06
	Fleroxacin	0.03–0.06	0.06	0.06
P. vulgaris (19)	E-4868	0.12–0.5	0.25	0.5
	Ciprofloxacin	0.015–0.06	0.03	0.06
	Ofloxacin	0.06–0.25	0.06	0.12
	Fleroxacin	0.06–0.12	0.12	0.12
P. mirabilis (19)	E-4868	0.25–1	0.5	1
	Ciprofloxacin	0.015–0.12	0.03	0.06
	Ofloxacin	0.06–0.5	0.12	0.5
	Fleroxacin	0.12–0.5	0.25	0.5
P. rettgeri (15)	E-4868	0.06–8	0.25	4
	Ciprofloxacin	0.015–1	0.03	0.5
	Ofloxacin	0.06–4	0.5	2
	Fleroxacin	0.06–4	0.12	2
P. stuartii (17)	E-4868	1–8	8	8
	Ciprofloxacin	0.12–8	2	8
	Ofloxacin	2–8	8	8
	Fleroxacin	1–8	8	8
M. morganii (20)	E-4868	0.06–1	0.25	0.5
	Ciprofloxacin	≤0.007–0.03	0.015	0.03
	Ofloxacin	0.03–0.12	0.06	0.12
	Fleroxacin	0.03–0.12	0.06	0.12
P. aeruginosa (20)	E-4868	0.5–4	1	2
	Ciprofloxacin	0.06–0.5	0.12	0.25
	Ofloxacin	1–4	1	4
	Fleroxacin	1–4	2	4
Clostridium spp. (9)[a]	E-4868	0.12–1	0.25	
	Ciprofloxacin	0.25–2	0.5	
Bacteroides spp. (19)[b]	E-4868	1–4	2	4
	Ciprofloxacin	2–16	4	8

[a] Includes four isolates of *C. perfringens*, one isolate of *C. sporogenes*, and four isolates of *Clostridium* spp.
[b] Includes 11 isolates of *B. fragilis*, 7 isolates of *Bacteroides* spp., and 1 isolate of *B. thetaiotaomicron*.

MIC_{90}s were 2- to 8-fold lower than those of ciprofloxacin and ofloxacin and between 2- and 32-fold lower than that for fleroxacin. The activity of E-4868 against members of the family *Enterobacteriaceae* was less than that of ciprofloxacin, except for *K. pneumoniae* and *Providencia stuartii*, for which the MIC_{90}s were the same as that of ciprofloxacin. For the other compounds, E-4868 MIC_{90}s were between two- and fourfold higher than those of fleroxacin and ofloxacin for *Serratia marcescens*, *P. vulgaris*, *Proteus mirabilis*, *Providencia rettgeri*, and *Morganella morganii*; equal for *Enterobacter cloacae*, *C. freundii*, and *Providencia stuartii*; and twofold lower for *E. coli*, *K. pneumoniae*, and *Salmonella typhi*.

Against *P. aeruginosa*, E-4868 inhibited 90% of the isolates at a concentration of 2 μg/ml; its activity was eightfold less than that of ciprofloxacin but twofold greater than those of fleroxacin and ofloxacin. Against anaerobes, E-4868 MICs were twofold lower than that of ciprofloxacin.

Factors affecting in vitro activity. (i) Effect of magnesium concentration. Table 2 shows the effect of increasing concentrations of Mg^{2+} on the MICs and MBCs of E-4868. MICs of E-4868 with MH broth supplemented with 1 mM Mg^{2+} remained unchanged, with the exception of the MIC for *S. aureus* HS-93. With MH broth supplemented with 5 mM Mg^{2+}, MICs increased two- to fourfold, and with medium with 10 mM Mg^{2+}, MICs increased four- to eightfold. The MBCs at all times were equal to or twofold higher than the MICs.

(ii) Effect of serum. In the medium containing 20% horse serum (Table 3), E-4868 was two- to fourfold less active against all strains tested. This decrease in the activity of E-4868 was 2- to 16-fold when tested in medium supplemented with 70% serum. In general, there was a proportional increase in MICs and MBCs.

(iii) Effect of urine. The effect of urine on the in vitro activity of E-4868 is shown in Table 3. The potency of E-4868 was decreased between 4- and 16-fold when tested in fresh urine at pH 7.2 compared with activities in broth, except against *P. vulgaris* HSP-99. At pH 5.5, MICs were unchanged, or increased at most fourfold, compared with the activities at pH 7.2.

Mouse protection studies. Table 4 shows the comparative therapeutic efficacy of E-4868 against lethal systemic infections with gram-positive cocci and gram-negative organisms

TABLE 2. Effect of Mg^{2+} on activity of E-4868

Organism	Concn (μg/ml) of E-4868 in:							
	MH broth alone		MH broth containing Mg^{2+} at:					
			1 mM		5 mM		10 mM	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus HS-93	0.03	0.06	0.06	0.12	0.12	0.25	0.25	0.5
E. coli HM-42	0.03	0.03	0.03	0.06	0.06	0.12	0.25	0.25
S. enteritidis HSP-928/F	0.12	0.12	0.12	0.25	0.25	0.25	0.5	1
K. pneumoniae HSP-30	0.06	0.06	0.06	0.06	0.12	0.25	0.25	0.25
C. freundii HSP-73	0.06	0.06	0.06	0.12	0.12	0.25	0.25	0.5
E. aerogenes HSP-145	0.12	0.12	0.12	0.12	0.25	0.25	0.5	0.5
P. vulgaris HSP-99	0.25	0.25	0.25	0.25	0.5	1	1	1
P. aeruginosa HS-116	1	2	1	2	2	4	4	8

in mice. E-4868 was efficacious against *S. aureus* HS-93, having an ED_{50} of 6.5 mg/kg. Against this strain, E-4868 was about seven times more effective than ciprofloxacin. E-4868 also demonstrated greater efficacy than ciprofloxacin against *S. pneumoniae* 84551. Against experimental infections with the gram-negative organisms *E. coli* HM-42 and *P. aeruginosa* HS-116, the protective effect of E-4868 was equal to that of ciprofloxacin.

Pharmacokinetics in mice. Data on the levels of E-4868 and ciprofloxacin in the blood in mice after oral administration of a 50-mg/kg dose are shown in Fig. 2. Absorption of E-4868 was very rapid, with a peak level in serum of 10.9 ± 2.5 μg/ml observed within 30 min; the level of ciprofloxacin at this time was 2.3 ± 2.0 μg/ml. The concentrations of E-4868 and ciprofloxacin in serum were 7.6 ± 1.8 and 1.2 ± 0.1 μg/ml, respectively, at 60 min postadministration and 7.8 ± 1.8 and 0.5 ± 0.3 μg/ml at 120 min postadministration. For E-4868, the level remained above 5.5 μg/ml for 240 min. The AUCs for E-4868 and ciprofloxacin were 28.4 and 2.3 μg · h/ml, respectively.

DISCUSSION

New fluoroquinolone antibacterial agents, such as norfloxacin, ciprofloxacin, ofloxacin, and others, have a broad spectrum of activity and have been used successfully in various kinds of infections in humans. However, their antibacterial activities are not optimal against certain pathogens such as gram-positive and anaerobic organisms.

The molecular structure of E-4868 is similar to that of other compounds described previously (9), except for a 2,4-difluorophenyl group at position 1 of the quinolone nucleus. The presence of a C-7 azetidin-1-yl is sufficient to account for the increased potency against gram-positive and anaerobic organisms compared with ciprofloxacin (8). In addition, the presence of a 2,4-difluorophenyl group also seems to increase this property. As a result, E-4868 showed four- to eightfold greater in vitro activity than ofloxacin or fleroxacin against staphylococci, streptococci, and enterococci. With the exceptions of methicillin-resistant *Staphylococcus* spp. and *E. faecalis*, E-4868 was two- to eightfold more potent than ciprofloxacin against gram-positive cocci. E-4868 showed twofold more potency than ciprofloxacin against isolates of the genera *Clostridium* and *Bacteroides*. Against gram-negative organisms, the in vitro activity of E-4868 was comparable to those of ofloxacin and fleroxacin but was eightfold lower than that of ciprofloxacin. However, members of the family *Enterobacteriaceae* were susceptible to E-4868; most of the enteric organisms were inhibited by ≤1 μg/ml. As with other fluoroquinolones, the addition of magnesium to the medium had a significant effect on the MICs and MBCs of E-4868. Its activity also decreased in the presence of horse serum or human urine at pH 5.5.

Pharmacokinetic studies with mice were carried out in order to assess the efficacy of E-4868. Ciprofloxacin was selected as the reference fluoroquinolone for in vivo studies because it is the most widely used of the new potent fluoroquinolones. Preliminary pharmacokinetic studies showed that after a single oral administration of 50 mg/kg,

TABLE 3. Effect of serum and urine on activity of E-4868

Organism	Concn (μg/ml) of E-4868 in:							
	Serum				Urine			
	20%		70%		pH 5.5		pH 7.2	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus HS-93	0.12	0.25	0.5	1	NVG[a]	NVG	NVG	NVG
E. coli HM-42	0.12	0.12	0.25	0.25	0.5	1	0.25	0.25
S. enteritidis HSP-928/F	0.25	0.5	0.25	0.5	2	2	0.5	0.5
K. pneumoniae HSP-30	0.25	0.5	0.5	2	2	2	1	2
C. freundii HSP-73	0.25	0.25	0.5	0.5	1	1	0.25	0.5
E. aerogenes HSP-145	0.25	0.5	1	2	4	4	2	2
P. vulgaris HSP-99	0.5	2	1	4	>64	ND[b]	32	32
P. aeruginosa HS-116	4	8	8	16	4	8	4	4

[a] NVG, no visible growth.
[b] ND, not determined.

TABLE 4. In vivo activities of E-4868 and ciprofloxacin against systemic infections in mice

Organism	Challenge dose (CFU/mouse)[a]	Test compound[b]	MIC (μg/ml)	MBC (μg/ml)	ED_{50} (mg/kg)[c]	
					Dose	95% confidence limit
S. aureus HS-93	8×10^9	E-4868	0.03	0.06	6.5	4.1–10.5
		Ciprofloxacin	0.12	0.25	44.6	13.6–145.9
E. coli HM-42	5.7×10^8	E-4868	0.03	0.03	3.9	2.0–7.4
		Ciprofloxacin	0.007	0.007	3.5	2.0–6.2
P. aeruginosa HS-116	3.3×10^8	E-4868	1	2	107.8	34.8–333.6
		Ciprofloxacin	0.06	0.12	93.2	32.2–269.4
S. pneumoniae 84551	1.2×10^6	E-4868	0.25	0.25	154.4	96.2–247.8
		Ciprofloxacin	1	2	>400	

[a] Mice were inoculated intraperitoneally with 0.5 ml of bacterial suspension, approximately five times the minimal lethal dose.
[b] Mice infected with *S. aureus*, *E. coli*, or *P. aeruginosa* were given a single oral dose immediately after bacterial challenge. Mice infected with *S. pneumoniae* were given four consecutive oral doses at 0, 6, 12, and 24 h postinfection.
[c] The ED_{50} was calculated by probit analysis (16); 95% confidence limits were calculated by the method of Litchfield and Wilcoxon (15).

E-4868 reached significantly higher concentrations in mouse serum than ciprofloxacin. Absorption of E-4868 was rapid, reaching a concentration in serum of 10.9 μg/ml within 30 min; in comparison, the concentration of ciprofloxacin was 2.3 μg/ml at this time. For E-4868, the concentration was above 5.5 μg/ml for 240 min; at this concentration, E-4868 was inhibitory against gram-positive anaerobic organisms and most gram-negative pathogenic organisms. In mice with systemic infections by the gram-positive cocci *S. aureus* HS-93 and *S. pneumoniae* 84551, the therapeutic effect of E-4868 was markedly greater than that observed with ciprofloxacin. In addition, E-4868 demonstrated a protective effect equal to that of ciprofloxacin in systemic infections with *E. coli* HM-42 and *P. aeruginosa* HS-116. Although the relative in vivo efficacy of E-4868 was greater than that of ciprofloxacin on the basis of in vitro activity, these results were consistent with the pharmacokinetic data obtained.

Our results suggest that, in general, E-4868 exhibits greater in vivo efficacy in mice than ciprofloxacin, possibly because of its better in vitro activity against gram-positive cocci and higher achievable levels in serum. Further studies are warranted in order to determine whether this activity can be demonstrated in other systems.

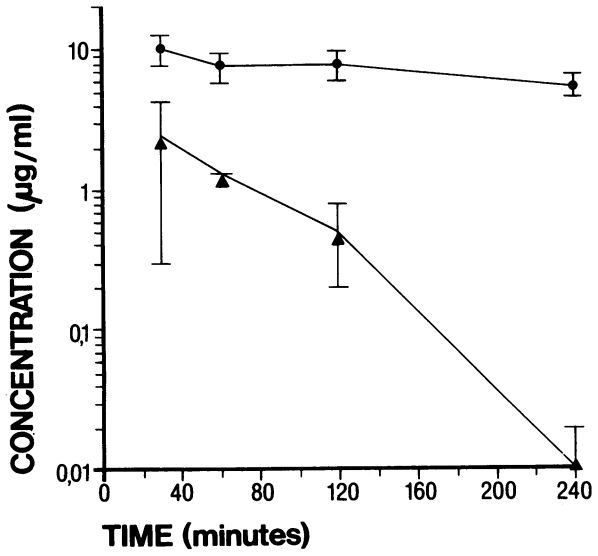

FIG. 2. Concentrations (mean ± standard deviation) of E-4868 (●) and ciprofloxacin (▲) in serum after a single 50-mg/kg oral dose in mice.

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