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Activity of E-3846, a New Fluoroquinolone, In Vitro and in Experimental Cystitis and Pyelonephritis in Rats

DOMINGO GARGALLO,[1]† MARIANO MOROS,[2] RAMÓN COLL,[2] MONTSERRAT ESTEVE,[2] JUAN PARÉS,[2] M. ANGELS XICOTA,[2] AND JESÚS GUINEA[1]*

Department of Microbiology, Division of Health Sciences, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona,[1] and Esteve S.A. Laboratories, Avda Mare de Deú de Montserrat 221, 08026 Barcelona,[2] Spain

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The in vitro antibacterial activity of E-3846, a new fluoroquinolone carboxylic acid derivative with a pyrrol ring substituent at position 7, was evaluated in comparison with norfloxacin and ciprofloxacin. E-3846 was more active than the reference quinolones against *Staphylococcus* species, including methicillin-resistant strains. E-3846 was similar to ciprofloxacin and more active than norfloxacin against *Streptococcus* (*Enterococcus*) *faecalis*. In general, E-3846 was more active than norfloxacin against members of the family *Enterobacteriaceae*, but less active than ciprofloxacin. For *Pseudomonas aeruginosa*, the MICs giving 90% inhibition for E-3846, norfloxacin, and ciprofloxacin were 2, 1, and 0.25 μg/ml, respectively. The activity of E-3846 increased at acid pH; in contrast, acid pH caused a pronounced decrease in the activity of norfloxacin and ciprofloxacin. In vivo, E-3846 demonstrated excellent therapeutic efficacy in treating experimental *S. faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* cystitis and pyelonephritis in rats.

The newer DNA gyrase subunit A inhibitors, such as amifloxacin (6, 14, 22), ciprofloxacin (1, 8, 23, 31), difloxacin (9, 26), enoxacin (4, 18, 20), irloxacin (5, 17), norfloxacin (3, 12, 13, 15), ofloxacin (10, 24), and perfloxacin (11, 27) are distinguished from earlier compounds such as nalidixic acid or oxolinic acid by modifications at positions 6, 7, and 8 on the ring structure. The addition of a fluorine atom to position 6 and modifications at position 7 resulted in an enormous increase in the antibacterial activity of these compounds (25).

E-3846 (6-fluoro-1-cyclopropyl-1,4-dihydro-4-oxo-7[pyrrol-1-yl]-quinolone-3-carboxylic acid [Fig. 1]) is a new fluoroquinolone antibacterial agent with a pyrrol ring at position 7 of the molecule. Other compounds with a 7-pyrrol ring substituent are irloxacin (17) and E-3604 (29).

In this report, we describe E-3846 and compare its in vitro antibacterial activity with those of norfloxacin and ciprofloxacin against a wide variety of organisms. We also examined the in vivo efficacy of E-3846 in the treatment of experimental urinary tract infections in rats.

MATERIALS AND METHODS

Antibacterial agents. E-3846 was synthesized at the Esteve S.A. Laboratories (Barcelona, Spain). Norfloxacin was obtained from Merck Sharp & Dohme (Rahway, N.J.), and ciprofloxacin was provided by Bayer A.G. (Wuppertal, Federal Republic of Germany). Antibacterial agent activities are expressed as micrograms of base per milliliter, and the antibiotic doses are shown as milligrams of base per kilogram.

Bacterial strains. The bacteria used in the first phase of this study were unique recent clinical isolates (only one isolate per patient) obtained from four geographically separate medical centers. The species represented in this collection are listed in Table 1. The effect of pH on the in vitro inhibitory activity of the antibacterial agents was studied with the following organisms: *Staphylococcus aureus* ATCC 5488/23, *Streptococcus* (*Enterococcus*) *faecalis* ATCC 10541, *Escherichia coli* ATCC 10536, *Proteus mirabilis* ATCC 4675, and *Pseudomonas aeruginosa* 25115. In the experimental urinary tract infections, five additional strains were used: *Streptococcus faecalis* U-8158; *E. coli* 24/16; *Proteus mirabilis* V-2835 and *Pseudomonas aeruginosa* S-1209, recently isolated from infected urine; and *Klebsiella pneumoniae* K66/O1, obtained from a laboratory stock culture used in previous studies.

In vitro studies. Growth-inhibitory activity was determined on solid or liquid medium by the antibiotic dilution technique. For the isolates listed in Table 1, comparative MICs were determined by the agar dilution method (28). Mueller-Hinton (MH) agar (Oxoid Ltd., London, England) was used for all testing except for *Streptococcus faecalis*, for which brain heart infusion agar (Oxoid) was used. Inocula were prepared by appropriate dilutions of overnight broth cultures. Plates were inoculated with a Steers-type multipoint inoculator which deposited approximately 10^5 CFU on the agar surface. The MICs were defined as the lowest concentration of antibacterial agent that inhibited develop-

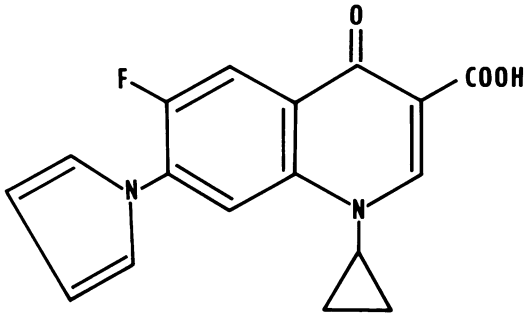

FIG. 1. Chemical structure of E-3846.

* Corresponding author.
† Present address: Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799.

TABLE 1. Antibacterial activity of E-3846 and reference compounds against clinical isolates

Organism (no. of isolates)	Antibacterial agent	MIC[a] (μg/ml)		
		Range	50%	90%
Staphylococcus aureus (95)	E-3846	0.03–0.5	0.03	0.03
	Norfloxacin	0.5–8.0	1.0	2.0
	Ciprofloxacin	0.12–2.0	0.5	1.0
Staphylococcus epidermidis (methicillin susceptible) (104)	E-3846	0.015–1.0	0.03	0.06
	Norfloxacin	0.25–16.0	1.0	2.0
	Ciprofloxacin	0.06–8.0	0.25	0.5
Staphylococcus epidermidis (methicillin resistant) (35)	E-3846	0.015–0.06	0.03	0.03
	Norfloxacin	0.5–2.0	1.0	2.0
	Ciprofloxacin	0.12–0.5	0.25	0.5
Streptococcus faecalis (23)	E-3846	0.5–1.0	0.5	1.0
	Norfloxacin	2.0–4.0	2.0	4.0
	Ciprofloxacin	0.5–1.0	0.5	1.0
Escherichia coli (58)	E-3846	0.03–2.0	0.12	0.12
	Norfloxacin	0.06–2.0	0.25	0.5
	Ciprofloxacin	0.007–0.5	0.06	0.06
Enterobacter spp. (10)	E-3846	0.12–0.5	0.25	0.5
	Norfloxacin	0.12–32.0	0.5	32.0
	Ciprofloxacin	0.007–4.0	0.06	2.0
Citrobacter freundii (6)	E-3846	0.03–0.25	0.12	
	Norfloxacin	0.12–0.5	0.25	
	Ciprofloxacin	0.003–0.03	0.015	
Klebsiella pneumoniae (36)	E-3846	0.12–8.0	0.25	4.0
	Norfloxacin	0.25–8.0	0.5	4.0
	Ciprofloxacin	0.06–2.0	0.25	1.0
Serratia spp. (12)	E-3846	0.06–0.5	0.25	0.5
	Norfloxacin	0.25–4.0	1.0	2.0
	Ciprofloxacin	0.03–0.5	0.25	0.5
Salmonella enteritidis (22)	E-3846	0.12–0.25	0.12	0.25
	Norfloxacin	0.25–1.0	0.5	0.5
	Ciprofloxacin	0.015–0.25	0.12	0.12
Salmonella spp. (10)	E-3846	0.12–0.25	0.12	0.12
	Norfloxacin	0.25–0.25	0.25	0.25
	Ciprofloxacin	0.015–0.03	0.015	0.03
Shigella spp. (6)	E-3846	0.03–1.0	0.12	
	Norfloxacin	0.12–2.0	0.12	
	Ciprofloxacin	0.003–0.25	0.003	
Proteus mirabilis (43)	E-3846	0.25–1.0	0.5	0.5
	Norfloxacin	0.06–0.5	0.12	0.25
	Ciprofloxacin	0.06–0.25	0.06	0.12
Proteus vulgaris (7)	E-3846	0.25–0.5	0.25	
	Norfloxacin	0.06–0.25	0.06	
	Ciprofloxacin	0.03–0.06	0.06	
Pseudomonas aeruginosa (28)	E-3846	0.5–2.0	2.0	2.0
	Norfloxacin	0.25–8.0	0.5	1.0
	Ciprofloxacin	0.12–2.0	0.12	0.25

[a] 50% and 90%, MICs for 50 and 90% of isolates, respectively.

ment of visible growth on agar. *Staphylococcus aureus* ATCC 25923, *Streptococcus faecalis* ATCC 29212, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883, *Salmonella typhimurium* ATCC 14028, *Proteus mirabilis* ATCC 7002, and *Pseudomonas aeruginosa* ATCC 27853 were used as control organisms.

The effect of pH on the activity of antibacterial agents and the MICs for the strains used to produce experimental urinary tract infections were determined by the standard tube macrodilution method (16). The effect of pH on the activity of E-3846, norfloxacin, and ciprofloxacin was determined in MH broth (Oxoid) adjusted to pH 4.8, 5.8, 6.8, 7.8,

or 8.8. An inoculum size of approximately 10^4 CFU/ml was used. Antibacterial agent-free tubes at different pHs were used as controls for growth.

To determine the MICs for the strains used to produce experimental urinary tract infections, inocula were prepared from overnight broth culture, diluted appropriately to 10^6 CFU/ml, and inoculated into serial twofold dilutions of E-3846 in MH broth. Subcultures were made for confirmation of purity and for quantitation of the inoculum sizes. The MICs in liquid medium were defined as the lowest concentration of antibacterial agent that inhibited development of visible growth in broth. MBCs were determined by quantitative subculture of 0.1 ml of broth from the control tube, the first tube containing growth, and from all tubes without visible growth on drug-free MH agar plates. The MBCs were defined as the lowest drug concentrations that killed ≥99.9% of the initial inoculum.

Urinary tract infections in rats. The bacterial strains used to establish infection were grown overnight on tryptic soy agar (Oxoid) and suspended in physiological saline solution (PSS) to the appropriate concentration. In each experiment, 40 female Wistar rats of strain CFHB (Interfauna U. K., Ltd., Huntington, England) weighing 200 to 250 g were used. Thirty animals were infected, and 10 were used as controls.

Under ether anesthesia (19), the rats were forced to void urine by gentle compression of the bladder through the external abdominal wall. A 1-ml portion of the bacterial suspension was immediately inoculated transurethrally into the bladder by using a sterile cannula (1.2 by 4.5 mm; Vasocan; B. Braun Melsurgen A.G.). In the control animals, sterile PSS replaced the bacterial suspension. After the inoculation, the external urethral meatus was blocked for 15 min by clamping to facilitate the ascent of the organisms from the bladder to the kidneys. The clamp was then removed, and the rats were kept in individual cages.

(i) Quantitation of infection level. A group of 10 rats was sacrificed 48 h postinfection (pretreatment control group), together with 5 control animals. The organs were removed and weighed under aseptic conditions. The bladder was rinsed with sterile PSS to remove any trace of urine. Afterwards, the bladder and each kidney were homogenized separately, serially 10-fold diluted in Ringer 1/4 solution, and cultured in duplicate on tryptic soy agar plates. The plates were incubated at 37°C for 18 to 20 h. When it was possible to obtain a sufficient quantity of urine, the samples were collected intraurethrally with a syringe and were cultured quantitatively as described above. Bacterial count was expressed as $\log_{10}$ CFU per gram of organ or $\log_{10}$ CFU per milliliter of urine. The lowest number of organisms detectable by this method was 50 CFU. The percentage of organs and urine samples infected, the mean of the $\log_{10}$ CFU, and the standard deviation of the mean of the positive cultures in 10 infected rats were calculated and accepted as the level of infection before treatment.

(ii) Treatment. Antibacterial therapy was started 2 days postinfection; a group of 10 rats was treated for 7 days with a single daily dose of E-3846. Experimental doses of 100 mg/kg in 1 ml of a 0.1% suspension of carboxymethyl cellulose were administered orally. Ten rats infected but left untreated were used as controls for the infection (posttreatment control group).

(iii) Determination of therapeutic efficacy. After 7 days of treatment and at least 18 h after administration of the last dose of antibacterial agent, the bladder, kidneys, and urine samples of treated and untreated rats, together with those from five animals inoculated with PSS, were cultured quantitatively as described above. The percentage of infected samples, the $\log_{10}$ CFU mean, and the standard deviation of the positive cultures in the treatment group were calculated and compared with the respective results of the posttreatment control group.

RESULTS

Activity against clinical isolates. Table 1 shows the susceptibility of the clinical isolates to E-3846 and to the reference fluoroquinolones. E-3846 was the most active of the tested compounds against *Staphylococcus* species, including methicillin-resistant isolates. For *Staphylococcus aureus*, norfloxacin and ciprofloxacin were 64 and 32 times less active than E-3846, respectively. For *Staphylococcus epidermidis*, norfloxacin and ciprofloxacin were 32 and 8 times less active, respectively. E-3846 was similar to ciprofloxacin against *Streptococcus faecalis* and four times more active than norfloxacin.

Against members of the family *Enterobacteriaceae*, E-3846 was generally more active than norfloxacin, but less active than ciprofloxacin. Against *Serratia* spp., E-3846 activity was similar to that of ciprofloxacin and four times more active than norfloxacin. E-3846 was also more active than norfloxacin against *E. coli*, *Enterobacter* spp., *Citrobacter freundii*, *Salmonella enteritidis*, and *Salmonella* spp. and similar to norfloxacin against *K. pneumoniae* and *Shigella* spp. E-3846 was less active than norfloxacin against *Proteus mirabilis* and *Proteus vulgaris*. Against *Pseudomonas aeruginosa*, E-3846 was two and four times less active than norfloxacin and ciprofloxacin, respectively.

Effect of pH on in vitro activity. The effect of pH on E-3846, norfloxacin, and ciprofloxacin activities against five tested strains is shown in Fig. 2. The MICs of E-3846 against *Staphylococcus aureus* ATCC 5488/23 and *Streptococcus faecalis* ATCC 10541 were 64 and 8 times lower at pH 4.8 than at pH 8.8, respectively. However, the MICs of norfloxacin and ciprofloxacin were 8 times higher against *Staphylococcus aureus* ATCC 5488/23 and 16 times higher against *Streptococcus faecalis* ATCC 10541 at pH 4.8 than at pH 8.8.

Against gram-negative bacteria, the MIC of E-3846 was four to eight times lower at pH 4.8 and four to eight times higher at pH 8.8 compared with the MIC at pH 6.8. The MIC of norfloxacin was 4 to 16 times higher at pH 4.8 and ranged from equal to four times lower at pH 8.8 when compared with the MIC at pH 6.8. The MIC of ciprofloxacin was eight times higher at pH 4.8 and two to four times lower at pH 8.8 when compared with the MIC at pH 6.8.

Efficacy of E-3846 against urinary tract infection in rats. Five models of cystitis and pyelonephritis were used to evaluate the therapeutic efficacy of E-3846. The MIC and MBC of E-3846 against the strains used for experimental infection are shown in Table 2. The experimental results of 150 rats inoculated with 10^8 to 10^9 CFU of bacteria are summarized in Table 3. None of the 10 healthy control rats inoculated with PSS in each experiment showed any bacterial growth in the kidneys, bladder, or urine.

The results indicate that practically all the animals developed urinary tract infection within 2 days of infection (pretreatment control groups) and that levels of infection in the untreated rats were maintained 9 days after inoculation (posttreatment control groups). However, a significant decrease in the percentage of positive cultures was observed in rats treated with E-3846. After antibacterial chemotherapy, the challenging organism was recovered from only one-

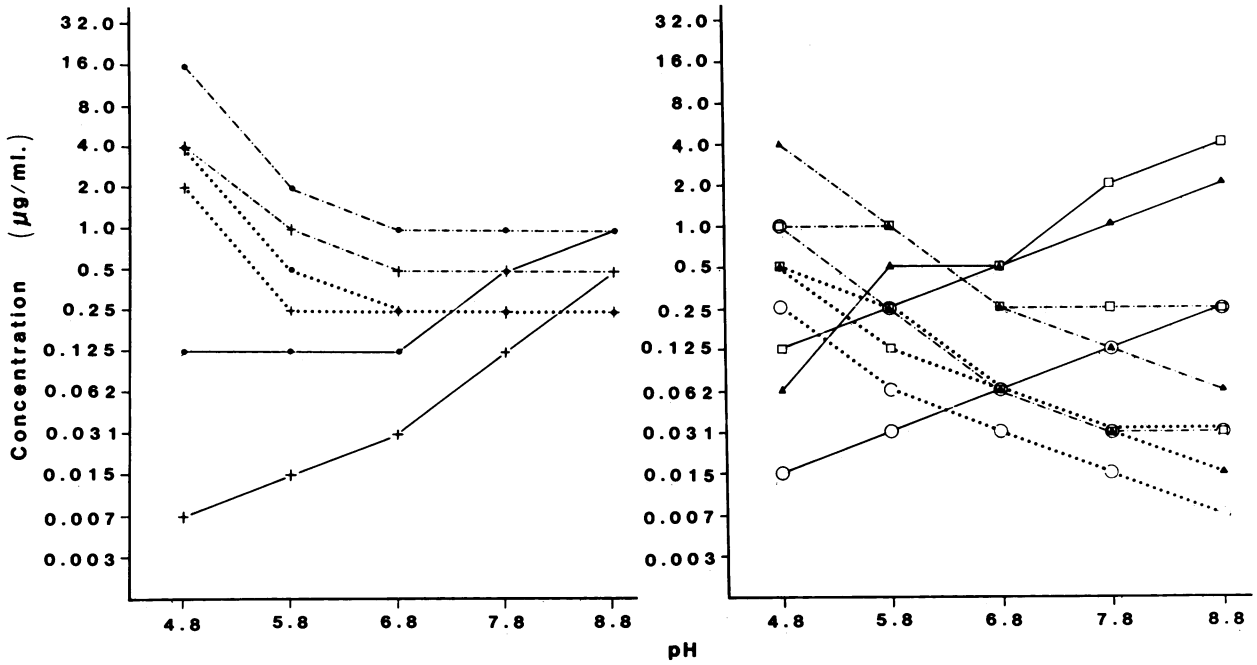

FIG. 2. Effect of pH on the activities of E-3846 (——), norfloxacin (—·—·—·), and ciprofloxacin (·····) in MH broth. Symbols: +, *Staphylococcus aureus* ATCC 5488/23; ●, *Streptococcus faecalis* ATCC 10541; ○, *E. coli* ATCC 10536; ▲, *Proteus mirabilis* ATCC 4675; □, *Pseudomonas aeruginosa* 25115.

fourth or fewer of the kidneys and from fewer than one-third of the bladders studied, with the exception of *Proteus mirabilis* V-2835, which was recovered from a larger number of organs. In the groups infected with *E. coli* 24/16, *K. pneumoniae* K66/O1, or *Proteus mirabilis* V-2835, there was also a quantitative decrease in the bacteria recovered from the organs of the treated rats in comparison with those from posttreatment control groups (Table 3).

The number of urine samples studied and the percentage of positive cultures are shown in Table 3. The relationship between the presence of bacteria in the urine and the existence of renal infection was absolute: all the rats with positive urine cultures showed renal infection.

DISCUSSION

E-3846 is a new synthetic fluoroquinolone with a potent broad-spectrum antibacterial activity. It is distinguished from other new agents such as norfloxacin and ciprofloxacin in that it contains a pyrrol ring instead of a piperazine ring at position 7 of the molecule (Fig. 1). E-3846 differs from the reference drugs in that it is approximately 32 to 64 times more active than norfloxacin and 8 to 32 times more active than ciprofloxacin against the common *Staphylococcus* species. E-3846 also differs from norfloxacin by being more active against *Streptococcus faecalis*. The staphylococci were generally inhibited by 0.03 to 0.06 μg of E-3846 per ml. Methicillin-resistant isolates of staphylococci were just as susceptible to E-3846 as the methicillin-susceptible isolates were (Table 1). In this study, the activity of E-3846 against most members of the family *Enterobacteriaceae* was superior to or of the same order of magnitude as the activity of norfloxacin and generally inferior to that of ciprofloxacin. Norfloxacin and ciprofloxacin were significantly more active against members of the *Enterobacteriaceae* than against *Staphylococcus* and *Streptococcus* species. Similar findings have been reported by other authors (1, 2, 21).

TABLE 2. MIC and MBC of E-3846 against the strains used for experimental urinary tract infection in rats

Bacterial strain	MIC (μg/ml)	MBC (μg/ml)
Streptococcus faecalis U-8158	0.5	0.5
Escherichia coli 24/16	0.06	0.06
Klebsiella pneumoniae K66/O1	0.5	1.0
Proteus mirabilis V-2835	0.25	0.5
Pseudomonas aeruginosa S-1209	2.5	5.0

E-3846 showed greater activity at acid pH, in contrast to norfloxacin and ciprofloxacin. The activity of norfloxacin and ciprofloxacin has been reported to be lowered by a decreasing pH (2, 7, 26). Among the fluoroquinolones studied, E-3846 was the most active at pH 4.8 against all tested gram-positive and gram-negative bacteria (Fig. 2). Other fluoroquinolones with a pyrrol ring substituent at position 7, irloxacin and E-3604, also show major activity at acid pH values (5, 29). It is possible that the pyrrol ring contributes to the excellent antibacterial activity of these compounds at acid pH. In general, the effect of pH should be considered when treating infections at sites where the pH may not be neutral. Since the pHs at many infections sites, such as abscesses, urinary tract, gall bladder, and prostate, are acidic, the activity of E-3846 may be greater in vivo than expected based simply on MICs. Thus, E-3846 would complement the spectrum of activity of other quinolones such as norfloxacin and ciprofloxacin, which could be better suited to the treatment of infections occurring in basic pH environments.

The in vivo evaluation of new antiinfective agents is generally done in systemically infected mice. These infections are characterized by an early and overwhelming septi-

TABLE 3. Therapeutic efficacy of E-3846 against experimental urinary tract infection in rats

Bacterial strain (infection dose, CFU/rat)	Sample	% Infected samples and viable bacterial counts[a]					
		Pretreatment control		E-3846 treated		Posttreatment control	
		% Infected[b]	Mean ± SD[c]	% Infected	Mean ± SD	% Infected	Mean ± SD
Streptococcus faecalis U-8158 (5.7×10^8)	Kidney	100	6.4 ± 1.0	25	5.6 ± 1.4[d]	100	6.4 ± 1.0
	Bladder	100	6.5 ± 1.1	30	5.3 ± 0.5[d]	100	6.1 ± 1.4
	Urine	100	5.9 ± 0.8 (7)	20	5.1 (5)	100	5.5 ± 1.2 (5)
Escherichia coli 24/16 (7.8×10^8)	Kidney	100	7.6 ± 1.6	20	4.0 ± 0.3[e]	100	7.8 ± 1.6
	Bladder	100	7.4 ± 1.3	30	3.8 ± 0.5[f]	100	6.6 ± 1.8
	Urine	100	9.1 ± 0.4 (6)	25	4.9 (4)	100	9.4 ± 0.4 (3)
Klebsiella pneumoniae K66/O1 (1.2×10^9)	Kidney	100	7.1 ± 0.6	15	3.3 ± 0.4[g]	95	6.2 ± 1.6
	Bladder	100	6.9 ± 0.9	30	3.5 ± 0.2[g]	100	6.2 ± 1.4
	Urine	100	9.1 ± 0.5 (5)	0	(4)	100	7.0 ± 1.3 (4)
Proteus mirabilis V-2835 (6.2×10^8)	Kidney	100	9.0 ± 0.7	35	4.0 ± 0.9[e]	85	7.9 ± 1.7
	Bladder	100	8.3 ± 0.3	40	3.9 ± 0.6[g]	90	7.3 ± 1.5
	Urine	100	9.9 ± 0.7 (4)	50	4.0 ± 0.7 (6)	100	8.8 ± 0.2 (4)
Pseudomonas aeruginosa S-1209 (9.3×10^7)	Kidney	95	7.3 ± 1.3	25	5.2 ± 1.9[f]	90	7.3 ± 1.6
	Bladder	100	5.5 ± 1.8	20	6.8 ± 1.5[d]	90	5.5 ± 1.5
	Urine	100	7.2 ± 0.3 (4)	33.3	6.0 ± 0.7 (6)	100	7.0 ± 0.6 (5)

[a] Pretreatment and posttreatment control groups were examined 2 and 9 days postinfection, respectively. In the treated group, antibacterial therapy was started 2 days postinfection and examined 7 days later. A total of 20 kidneys and 10 bladders were studied in each group. Numbers in parentheses represent urine samples studied.

[b] Percentage of positive cultures. The lowest number of organisms detectable by the method used was 50 CFU per gram of organ or milliliter of urine. None of the healthy control rats inoculated with PSS showed any bacterial growth in the kidneys, bladder, or urine.

[c] Values represent the mean $\log_{10}$ CFU per gram of organ or $\log_{10}$ CFU per milliliter urine ± standard deviation of the positive cultures.

[d] P is not significant for treated group versus posttreatment control group.

[e] $P < 0.001$ for treated group versus posttreatment control group.

[f] $P < 0.05$ for treated group versus posttreatment control group.

[g] $P < 0.01$ for treated group versus posttreatment control group.

cemia which bears little resemblance to the type of localized infections usually caused by these organisms in humans. The therapeutic efficacy of E-3846 was studied in the treatment of urinary tract infection in rats, considering the advantages of localized models of chemotherapy (30). The results of this study demonstrate that orally administered E-3846 is significantly effective in the treatment of cystitis and pyelonephritis caused by important urinary tract pathogens (Table 3). E-3846 showed excellent antibacterial activity against *Streptococcus faecalis* and gram-negative bacteria, such as members of the *Enterobacteriaceae*, including *K. pneumoniae*, and also against *Pseudomonas aeruginosa*. Although E-3846 did not show great activity against *K. pneumoniae* and *Pseudomonas aeruginosa* in vitro, it was extremely effective in vivo in eradicating these organisms from the bladder and kidneys. However, despite the enhanced activity of E-3846 against *Proteus mirabilis* in vitro, its efficacy in vivo was inferior in comparison with its effectiveness against other organisms, possibly because *Proteus mirabilis* is a urea-splitting organism.

In summary, the potent broad-spectrum activities (especially against gram-positive bacteria) as well as the therapeutic efficacy in treating urinary tract infections and the possibility of using E-3846 in the treatment of other localized and systemic infections make this compound a potentially useful therapeutic agent.

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