IN VITRO AND *IN VIVO* ANTIBACTERIAL ACTIVITY OF KY-109, A NEW ORALLY ACTIVE CEPHALOSPORIN

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The *in vitro* and *in vivo* antimicrobial potencies of KY-109, a pro-drug of KY-087, were compared with those of amoxicillin, cephalexin (CEX), and cefaclor (CCL). The following results were obtained.

KY-087, which is the active form of KY-109, had broad antimicrobial spectrum against Gram-positive and Gram-negative organisms, but showed low antimicrobial activity against *Enterobacter* sp., *Serratia*, and *Pseudomonas* sp. The antimicrobial activities of KY-087 against clinically isolated Gram-positive organisms were superior to those of CEX and CCL. The antimicrobial activities of KY-087 against Gram-negative organisms, such as *Enterobacter* sp., *Serratia*, and *Pseudomonas* sp., were less active. KY-087 showed dose-related bactericidal activity against *Staphylococcus aureus* and *Escherichia coli*. The therapeutic efficacy of KY-109 against experimental intraperitoneal infections caused by Gram-positive and Gram-negative organisms in mice was comparable to that of CEX but inferior to that of CCL. In experimental granuloma pouch models in rats and kidney infection in rabbits, therapeutic efficacy of KY-109 was either comparable or superior to that of CEX and CCL.

KY-087, 7-(D-mandelamido)-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylic acid, a new cephalosporin from Kyoto Pharmaceutical Co., Ltd., has a broad spectrum of antibacterial activity *in vitro*[1]. However, KY-087 after administered orally, is poorly absorbed from the gastrointestinal tract owing probably to its low lipophilicity.

KY-109, (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl 7-[D-*O*-(L-alanyl)mandelamido]-3-[[(5-methyl-1,3,4-thiadiazol-2-yl)thio]methyl]-3-cephem-4-carboxylate hydrochloride, is synthesized as a bifunctional pro-drug designed to improve the oral absorption of the parent drug (KY-087).

In this paper, the antimicrobial potencies of KY-109 are compared with those of amoxicillin (AMPC), cephalexin (CEX), and cefaclor (CCL) *in vitro* and *in vivo*.

Materials and Methods

Organism

Clinically isolated strains used in this study were obtained from several hospitals. The other strains used were stock cultures from our laboratory.

Antimicrobial Agents

The antimicrobial agents were supplied by the following organizations: KY-087 and KY-109, Kyoto Pharmaceutical Co., Ltd., AMPC, Fujisawa Pharmaceutical Co., Ltd.; and CEX and CCL, Shionogi & Co., Ltd. Solutions of the antimicrobial agents were freshly prepared each day.

Susceptibility Studies

Antibiotic susceptibility was determined by an agar dilution technique using sensitivity test agar (Eiken). This medium was supplemented with 10% defibrinated horse blood for Streptococci and with 5% Fildes enrichment (Difco) for *Haemophilus influenzae*. All strains except Streptococci and

H. influenzae were grown overnight in sensitivity test broth (Eiken). Streptococci and *H. influenzae* were grown overnight in sensitivity test broth with 10% horse serum and with 5% Fildes enrichment, respectively. Overnight cultures were diluted 100-fold in phosphate-buffered saline (pH 7.2) and applied by means of the multiple inocula replicator to the surface of agar plates containing 2-fold dilutions of antibiotics. The MIC was defined as the lowest concentration of antibiotic inhibiting growth after overnight incubation at 37°C.

Time-kill Curve Studies

The bactericidal activity of KY-087 was measured in nutrient broth (Nissui). In brief, stock culture was grown for 18 hours at 37°C in nutrient broth and subsequently diluted with the same medium to a cell density of approximately 10^6 cfu/ml. Aliquots of 9.0 ml of this inoculated medium were transferred to flasks. The flasks were placed in a water bath at 37°C and 1.0 ml of medium containing 10 times the desired initial concentration of antibiotics was added. The flasks were incubated at 37°C, 0.5 ml aliquots were removed at intervals, diluted in saline, and plated for viable cell count determination. Colonies were counted after 24 hours of incubation at 37°C and the number of viable bacteria in the samples was estimated.

Animal Studies

Acute Systemic Infection: Male *dd*Y mice weighing 17~19 g were infected intraperitoneally with 0.5 ml of a bacterial suspension in phosphate-buffered saline (Streptococci) or in 3% hog gastric mucin (Orthana Kemisk Fabrik A/S, other strains). The antibiotic doses were contained in 0.2 ml and administered by gavage 2 hours after challenge. In each test, 8 mice were treated at each dose level, and survival ratios were determined 7 days after infection. The ED_{50} were estimated by the LITCHFIELD-WILCOXON method[2]. In addition, the antibiotics were administered in single doses to 5 mice infected with *Escherichia coli* 444, and concentrations of antibiotics in serum and peritoneal exudate were determined by microbiological assay with *Bacillus subtilis* ATCC 6633 as the test organism.

Granuloma Pouch Models: Under ether anaesthesia, male Wistar rats weighing 160~180 g were injected subcutaneously on the back with 20 ml of germ-free air sterilized by membrane filter (0.45 μm) by means of a syringe with a 25-gauge needle. An inflammatory reaction was induced by injecting 1 ml of sterile 1% croton oil solution in olive oil into the formed air pouch with a 23-gauge needle. After 48 hours, the air was removed with a 25-gauge needle. On the 7th day the animals weighed 200~220 g and bore granuloma pouches with *ca.* 12 ml exudate to which bacterial inocula to be described were injected with a 25-gauge needle[3]. Each drug was administered orally 1 hour after injection. At intervals, samples of exudate were taken from the pouches to determine the numbers of viable bacteria. In addition, the antibiotics were administered in single doses to 4 rats infected with *E. coli* 444, and concentrations of antibiotics in pouch exudate were determined by microbiological assay.

Ascending Urinary Tract Infection[4]: Male rabbits weighing *ca.* 2 kg were deprived of water for 15 hours. The abdominal wall and the left flank were shaved before anaesthesia with pentobarbital. The rabbits were anaesthetized and the abdominal wall was opened through a midline incision (approximately 5 cm in length). The left ureter was ligatured loosely with silk suture. 0.1 ml of nutrient broth containing *E. coli* 444 diluted from an overnight culture was inoculated directly into the ureter using a syringe with a 25-gauge needle. The abdominal wall was closed in a single layer with ten silk stitches. Eighteen hours after inoculation, the antibiotics were administered orally. Therapeutic efficacies were assessed in terms of the numbers of bacteria found in the kidney. The numbers of bacteria in the kidneys were measured at 72 hours after inoculation.

Results

In Vitro Susceptibility Studies

Antibacterial Spectrum

To assess the antibacterial spectrum of KY-087, its antibacterial activities against the typical

Table 1. Antibacterial spectrum of Gram-positive bacteria (10^6 cells/ml).

Organism	MIC (μg/ml)			
	KY-087	CEX	CCL	AMPC
Staphylococcus aureus 209P-JC	0.2	3.13	0.78	0.1
S. aureus Smith	0.2	1.56	0.78	0.1
S. aureus Neumann	≦0.006	≦0.006	≦0.006	≦0.006
S. aureus Terashima	0.39	12.5	3.13	0.2
S. aureus E-46	0.2	1.56	0.78	0.1
S. aureus No. 80	0.39	1.56	1.56	1.56
S. epidermidis KC-1	0.78	6.25	1.56	0.39
Streptococcus pyogenes S 23	0.05	0.78	0.2	0.012
S. pyogenes Cook	0.05	0.78	0.2	0.012
S. pyogenes C 203	0.012	0.2	0.1	≦0.006
S. pneumoniae I	0.05	3.13	0.39	0.012
S. pneumoniae II	0.05	1.56	0.39	0.012
S. pneumoniae III	0.05	3.13	0.39	0.012
Viridans group *Streptococcus*	50	>100	25	0.39
Enterococcus faecalis KC-1	50	>100	25	0.39
Corynebacterium diphtheriae KC-1	0.2	0.78	0.39	0.2
Micrococcus luteus ATCC 9341	0.05	0.05	0.025	≦0.006
Bacillus subtilis ATCC 6633	0.025	0.78	0.1	0.012
B. anthracis KC-1	0.1	1.56	0.39	0.012

Abbreviations: CEX, cephalexin; CCL, cefaclor; AMPC, amoxicillin.

Table 2. Antibacterial spectrum of Gram-negative bacteria (10^6 cells/ml).

Organism	MIC (μg/ml)			
	KY-087	CEX	CCL	AMPC
Escherichia coli NIHJ JC-2	1.56	6.25	1.56	6.25
E. coli NIH	0.39	6.25	1.56	3.13
Citrobacter freundii NIH 10018-68	12.5	100	25	100
Salmonella typhi T-287	0.1	1.56	0.2	0.2
S. typhi O 901	0.2	3.13	0.78	0.39
S. paratyphi-A	0.2	6.25	0.78	0.39
S. paratyphi-B	0.2	3.13	0.39	0.39
S. enteritidis KC-1	0.39	3.13	0.78	0.39
Shigella dysenteriae EW 7	1.56	6.25	1.56	3.13
S. flexneri 2a EW 10	0.78	6.25	0.78	1.56
S. boydii EW 28	0.39	6.25	1.56	3.13
S. sonnei EW 33	0.39	3.13	1.56	3.13
Klebsiella pneumoniae NCTC 9632	0.78	6.25	0.78	50
Enterobacter aerogenes NCTC 10006	12.5	>100	100	>100
E. cloacae NCTC 9394	12.5	>100	100	>100
Hafnia alvei NCTC 9540	12.5	>100	25	>100
Serratia marcescens IFO 3736	>100	>100	>100	25
Proteus mirabilis 1287	0.39	6.25	0.78	0.39
P. vulgaris OX-19	3.13	12.5	12.5	6.25
P. inconstans NIH 118	0.39	12.5	3.13	50
P. rettgeri NIH 96	0.05	6.25	0.78	0.2
Morganella morganii Kono	12.5	>100	>100	>100
Pseudomonas aeruginosa NCTC 10490	>100	>100	>100	>100
P. cepacia ATCC 25416	>100	>100	>100	>100
Xanthomonas maltophilia ATCC 13637	>100	>100	>100	>100
Acinetobacter calcoaceticus IFO 12552	>100	100	100	100

Abbreviations: See footnote in Table 1.

Table 3. Comparative antimicrobial potencies of KY-087 and reference compounds against various bacteria.

Organism	Drug	MIC range (μg/ml)	MIC_{50}	MIC_{90}
Staphylococcus aureus (41)	KY-087	0.78~50	0.2	0.2
	AMPC	0.025~1.56	0.1	0.39
	CEX	0.2~6.25	1.56	1.56
	CCL	0.1~6.25	0.78	1.56
S. epidermidis (35)	KY-087	0.1~6.25	0.39	1.56
	AMPC	0.05~50	0.39	12.5
	CEX	0.78~100	3.13	50
	CCL	0.39~50	0.78	25
Streptococcus pyogenes (26)	KY-087	0.025~0.2	0.025	0.05
	AMPC	0.006~0.025	0.012	0.025
	CEX	0.2~0.39	0.39	0.39
	CCL	0.1~0.78	0.20	0.39
Escherichia coli (42)	KY-087	0.2~100	3.13	12.5
	AMPC	0.78~>100	>100	>100
	CEX	3.13~100	6.25	12.5
	CCL	0.78~100	1.56	3.13
Klebsiella pneumoniae (42)	KY-087	0.78~50	3.13	50
	AMPC	25~>100	100	>100
	CEX	3.13~12.5	6.25	6.25
	CCL	0.39~12.5	0.78	3.13
Enterobacter cloacae (22)	KY-087	3.13~>100	6.25	>100
	AMPC	50~>100	>100	>100
	CEX	6.25~>100	>100	>100
	CCL	6.25~>100	50	>100
E. aerogenes (22)	KY-087	3.13~>100	12.5	>100
	AMPC	3.13~>100	>100	>100
	CEX	12.5~>100	>100	>100
	CCL	3.13~>100	>100	>100
Serratia marcescens (43)	KY-087	12.5~>100	>100	>100
	AMPC	12.5~>100	>100	>100
	CEX	100~>100	>100	>100
	CCL	50~>100	>100	>100
Proteus vulgaris (37)	KY-087	1.56~>100	50	>100
	AMPC	1.56~>100	>100	>100
	CEX	3.13~>100	>100	>100
	CCL	0.78~>100	>100	>100
P. mirabilis (37)	KY-087	0.39~25	6.25	12.5
	AMPC	0.39~>100	0.78	0.78
	CEX	0.39~12.5	12.5	12.5
	CCL	0.39~3.13	0.78	1.56
Morganella morganii (28)	KY-087	0.78~>100	6.25	>100
	AMPC	50~>100	>100	>100
	CEX	50~>100	>100	>100
	CCL	25~>100	>100	>100
Providencia rettgeri (16)	KY-087	0.2~>100	25	>100
	AMPC	0.2~>100	>100	>100
	CEX	0.05~>100	6.25	>100
	CCL	0.78~>100	>100	>100
Haemophilus influenzae (41)	KY-087	0.2~3.13	0.39	0.78
	AMPC	0.1~6.25	0.39	0.39
	CEX	0.78~25	6.25	12.5
	CCL	0.39~6.25	1.56	1.56

Table 3. (Continued)

Organism	Drug	MIC range (μg/ml)	MIC_{50}	MIC_{90}
Acinetobacter calcoaceticus (21)	KY-087	1.56~100	50	100
	AMPC	1.56~50	12.5	50
	CEX	25~>100	>100	>100
	CCL	6.25~100	25	100
Pseudomonas aeruginosa (17)	KY-087	>100	>100	>100
	AMPC	>100	>100	>100
	CEX	>100	>100	>100
	CCL	>100	>100	>100

Abbreviations: See footnote in Table 1.
( ): No. of strains.

laboratory strains were compared with those of AMPC, CEX, and CCL (Tables 1 and 2). The MICs of KY-087 ranged from 0.025 to 6.25 μg/ml against Staphylococci, Streptococci, *E. coli*, *Salmonella* sp., *Shigella* sp., and *Klebsiella pneumoniae*. KY-087 did not inhibit *Citrobacter*, *Enterobacter*, *Serratia*, *Proteus vulgaris*, *Morganella morganii*, *Pseudomonas* sp., and *Acinetobacter* at a concentration of 100 μg/ml. The antibacterial spectrum and potency of KY-087 were considered to be slightly superior to those of CEX and CCL.

Susceptibility of Clinical Isolates

The *in vitro* antimicrobial potencies of KY-087 were compared with those of AMPC, CEX, and CCL against clinical isolates of 470 organisms. The results of these comparisons are shown in Table 3. The MICs against 90% of the strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, and *H. influenzae* tested ranged from 0.05 to 0.78 μg/ml for KY-087, from 0.025 to 3.13 μg/ml for AMPC, from 0.39 to 12.5 μg/ml for CEX, and from 0.39 to 6.25 μg/ml for CCL. KY-087 was generally more potent than reference compounds against Gram-positive cocci; however, against Gram-negative bacteria KY-087 was generally less active. KY-087 displayed poor activity against *Serratia marcescens* and *Pseudomonas aeruginosa*.

Time-kill Curve Studies

The extent and rapidity of killing by KY-087, CEX, and CCL were determined by adding each antimicrobial agent to logarithmic-phase cultures of *S. aureus* and *E. coli*. The viable counts determined 1 to 4 hours after addition of each drug are shown in Figs. 1 and 2. The viable count of *S. aureus* was reduced approximately 99% within 4 hours by two times the MIC of KY-087, CEX, or CCL. In *E. coli*, the rapid killing effect was observed with KY-087 and CCL, which reduced the viable count approximately 99.9% within 2 hours.

Animal Studies

Acute Systemic Infection

The potencies of KY-109, CEX, and CCL in several mouse protection tests are shown in Table 4. The ED_{50} values of KY-109 ranged from 1.56 to 155.56 mg/kg. In general, KY-109 was equal to or slightly more potent than CEX. KY-109 was 2.4- to 20-fold less effective than CCL when administered orally against all tested strains except *S. pneumoniae*. The mean concentrations of KY-109, CEX, and CCL in serum and peritoneal exudate after oral administration of a 50-mg/kg dose to mice infected with *E. coli* 444 are shown in Table 5. The concentrations of KY-109 in serum fell rapidly from

Fig. 1. Effect of KY-087 (A), cephalexin (B), and cefaclor (C) on the viability of *Staphylococcus aureus* Smith.

■ No drug, ○ 0.1 μg/ml, △ 0.2 μg/ml, ▽ 0.39 μg/ml, ● 0.78 μg/ml, ▲ 1.56 μg/ml, ▼ 3.13 μg/ml.

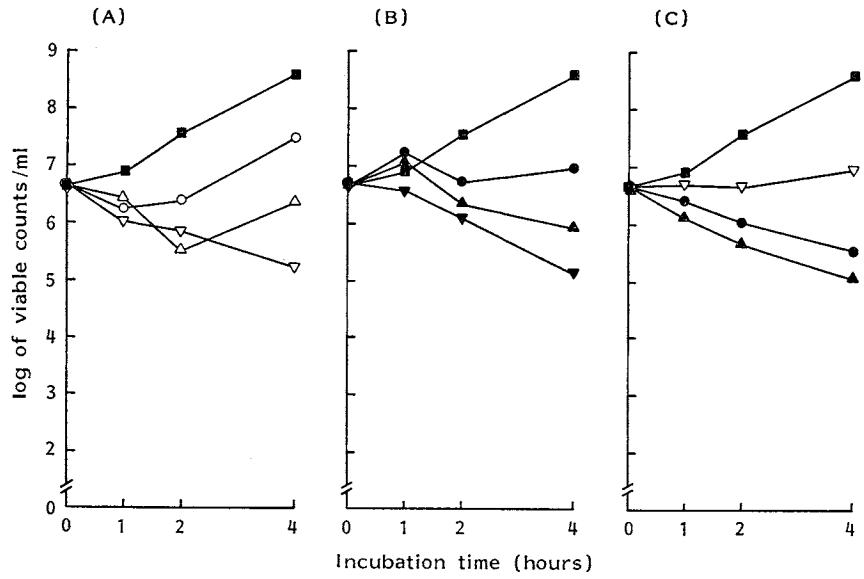
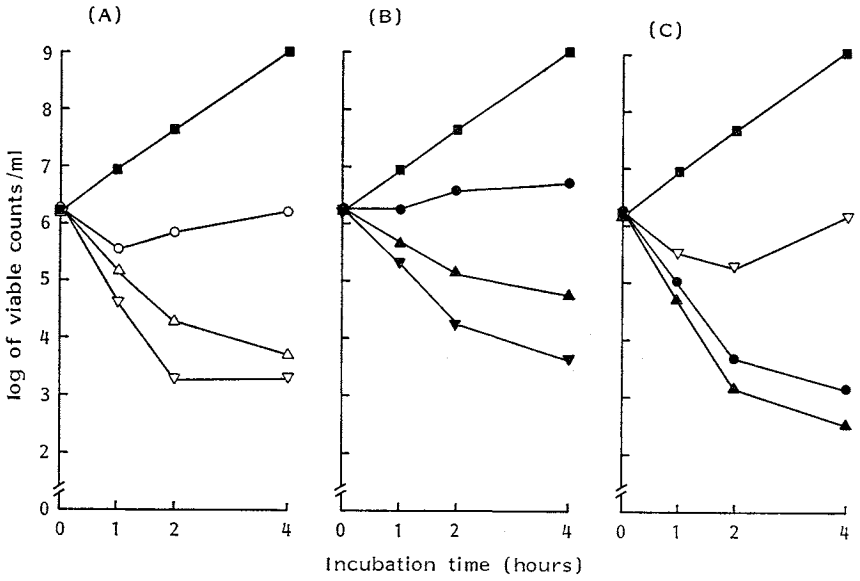


Fig. 2. Effect of KY-087 (A), cephalexin (B), and cefaclor (C) on the viability of *Escherichia coli* 444.

■ No drug, ○ 0.2 μg/ml, △ 0.39 μg/ml, ▽ 0.78 μg/ml, ● 1.56 μg/ml, ▲ 3.13 μg/ml, ▼ 6.25 μg/ml.



18.8 μg/ml 30 minutes after administration to 8.4 μg/ml after 60 minutes and to 1.7 μg/ml after 240 minutes. The mean peak concentrations of 26.5 μg/ml CEX and 7.1 μg/ml CCL were obtained 30 minutes after administration. In peritoneal exudate, mean peak concentrations of 2.3 μg/ml KY-109, 10.9 μg/ml CEX, and 6.2 μg/ml CCL were obtained 30 minutes after administration. The area under the curve (AUC) of KY-109, CEX, and CCL was 4.4, 18.2, and 10.5 μg·hour/ml, respectively.

Table 4. Protective effect of KY-109, cephalexin (CEX), and cefaclor (CCL) on experimental infections with a variety of bacteria in mice.

Bacterial strain	Infective dose (cfu/mouse) ($\times LD_{50}$)	Test compound	ED_{50} (mg/kg) (95% confidence limits)	MIC (μg/ml)
Staphylococcus aureus Smith	6.5×10^5 (25)	KY-109	1.56 (1.28~1.89)	0.2
		CEX	0.28 (0.21~0.37)	1.56
		CCL	0.14 (0.11~0.18)	0.78
Streptococcus pneumoniae III	6.5×10 (10)	KY-109	10.00 (7.67~13.00)	0.05
		CEX	72.22 (55.44~94.11)	3.13
		CCL	22.22 (14.17~34.83)	0.39
S. pyogenes C 203	4.0×10^3 (130)	KY-109	4.72 (3.67~6.11)	0.012
		CEX	3.61 (3.00~4.33)	0.2
		CCL	1.00 (0.78~1.33)	0.1
Escherichia coli 444	5.0×10^4 (10)	KY-109	4.72 (3.56~6.28)	0.39
		CEX	6.67 (4.78~9.28)	3.13
		CCL	1.94 (1.44~2.56)	1.56
Klebsiella pneumoniae KC-1	3.5×10^2 (250)	KY-109	155.56 (118.89~203.33)	0.78
		CEX	111.11 (92.22~133.33)	6.25
		CCL	11.67 (8.33~16.11)	0.39
Proteus mirabilis 434	4.5×10^6 (8)	KY-109	40.00 (23.89~66.67)	3.13
		CEX	51.67 (30.00~89.44)	12.5
		CCL	2.00 (1.17~3.44)	1.56

Table 5. Serum and peritoneal exudate levels of KY-109, cephalexin (CEX), and cefaclor (CCL) after administration in mice infected with *Escherichia coli* 444.

Sample	Compound	Time after administration (minutes)				AUC[b]
		30	60	120	240	
Serum	KY-109	18.8±1.37[a]	8.4±0.71	3.3±0.67	1.7±0.55	22.4
	CEX	26.5±1.55	15.1±1.40	7.6±0.80	2.3±0.41	38.3
	CCL	7.1±0.66	3.3±0.47	1.6±0.21	0.7±0.06	9.1
Peritoneal exudate	KY-109	2.3±0.28	2.1±0.20	0.9±0.22	0.3±0.32	4.4
	CEX	10.9±1.50	8.3±0.47	3.5±0.56	2.6±1.41	18.2
	CCL	6.2±0.66	4.8±0.43	2.0±0.27	1.1±0.26	10.5

Each drug: 50 mg/kg.
[a] μg/ml (±SE).
[b] μg·hour/ml.

Granuloma Pouch Models

With an inoculum of 10^6 cfu, the MIC of KY-087 was 0.2 μg/ml against *S. aureus* Smith and 0.39 μg/ml against *E. coli* 444. KY-087 was 4- to 8-fold more active than CEX and CCL. The efficacies of a single oral dose of KY-109, CEX, or CCL on the bacterial counts in pouch exudate of rats are shown in Figs. 3 and 4. Against *S. aureus*, KY-109 and CCL caused reductions of bacterial cells, with clearance of the organisms from the pouch at 60 mg/kg. CEX, however, was ineffective at the same dose. Against *E. coli*, KY-109, CEX, and CCL caused reductions in the viable count in the pouch within 4 hours, and after 12 hours, bacterial regrowth was observed. However, KY-109 caused suppression of regrowth in the pouch at 24 hours after administration. The mean concentration of KY-109, CEX, and CCL in pouch exudate after oral administration of a 120-mg/kg dose to rats infected with *E. coli* 444 are shown in Table 6. The mean peak concentrations of 2.20 μg/ml

Fig. 3. Therapeutic efficacy of KY-109 (○), cephalexin (△), and cefaclor (▲) on number of bacteria in the granuloma pouch of rat infected with *Staphylococcus aureus* Smith.

● No drug. Each drug: 60 mg/kg. $n=3$.

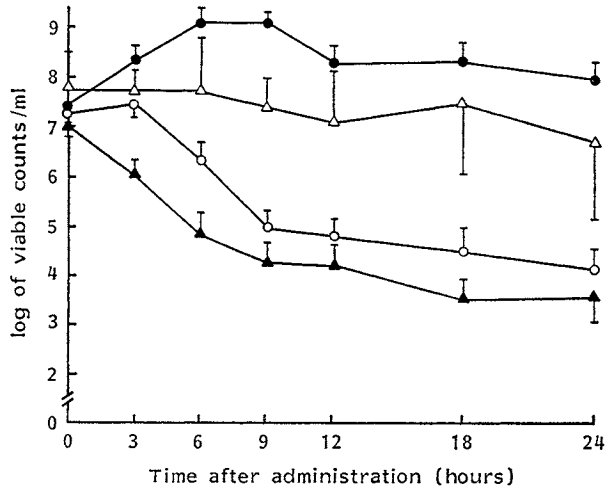
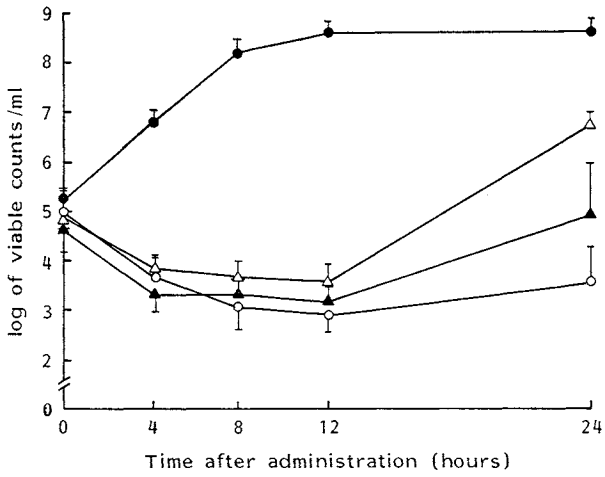


Fig. 4. Therapeutic efficacy of KY-109 (○), cephalexin (△), and cefaclor (▲) on number of bacteria in the granuloma pouch of rat infected with *Escherichia coli* 444.

● No drug. Each drug: 120 mg/kg. $n=4$.



KY-109 were obtained 8 hours after administration. KY-109, which corresponds to the MIC level against *E. coli* 444, was detectable in the pouch exudate at 24 hours after administration. With CEX and CCL, the mean peak level was obtained 2 hours after administration and there was an initial rapid distribution phase followed by a gradual and steady decline.

Ascending Urinary Tract Infection

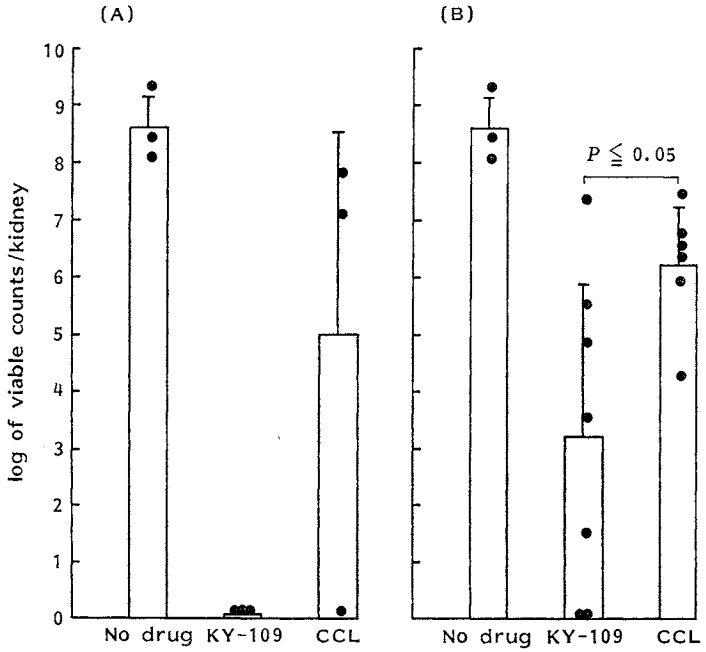
A rabbit model for pyelonephritis was used to evaluate the potency of KY-109. This model infection is difficult to treat because organisms multiply in kidney tissues. The kidneys of *E. coli*-infected rabbits contained approximately 3×10^4 cfu 18 hours before treatment. The effect of oral treat-

Table 6. Pouch exudate levels of KY-109, cephalexin (CEX), and cefaclor (CCL) after administration in rats infected with *Escherichia coli* 444.

Compound	Time after administration (hours)						
	0.5	1	2	4	6	8	24
KY-109	0.32±0.32[a]	0.88±0.34	1.48±0.54	1.37±0.51	1.47±0.52	2.20±0.33	0.37±0.12
CEX	0.80±0.47	3.96±1.14	8.82±1.00	8.68±0.74	6.26±0.94	4.79±0.85	N.D.
CCL	0.32±0.14	1.65±0.46	3.19±0.62	2.61±0.69	1.70±0.34	1.55±0.53	N.D.

Each drug: 120 mg/kg. $n=4$.
[a] μg/ml (±SE).
N.D.: Not detected.

Fig. 5. Therapeutic efficacy of KY-109 and cefaclor (CCL) on number of bacteria in the kidney of rabbit infected with *Escherichia coli* 444.
(A) 150 mg/kg(bid) ×2 days, (B) 75 mg/kg(bid) ×2 days.



ment for 2 days on bacteria in the kidneys is shown in Fig. 5. KY-109 was superior to CCL in their ability to clear the kidneys of *E. coli*.

Discussion

KY-087 had a broad spectrum of antibacterial activity. The *in vitro* activity of KY-087 against Gram-positive bacteria was slightly superior to that of CEX and CCL but slightly lower than that of AMPC. KY-087 showed good activity against *E. coli*, *Salmonella* sp., and *Shigella* sp. However, it was less active against other Gram-negative bacteria such as *Enterobacter* sp., *Serratia*, and *Pseudomonas* sp.

KY-087 showed bactericidal activity at concentrations of MIC or more against *S. aureus* and *E. coli*. This activity was similar to that of CCL and slightly superior to that of CEX.

Against systemic infections, the activity of KY-109 was almost similar to that of CEX but inferior to that of CCL. After a single oral administration of KY-109 to mice, its peak level and AUC in

serum were 18.8 μg/ml and 22.4 μg·hour/ml, respectively. However, the peak level and AUC of KY-109 in peritoneal exudate were lower than those of CEX and CCL. This suggested that the poor chemotherapeutic efficacy of KY-109 in mice might be a result of its low peritoneal exudate levels and AUC. However, KY-109 has good pharmacological properties in various animals such as rat and rabbit.

In rat and rabbit models, the therapeutic efficacies of KY-109 were equivalent or superior to those of CCL and were superior to those of CEX. Thus, the greater *in vivo* activities of KY-109 in rats and rabbits might be reasonably attributed to its slightly greater bactericidal activity and good absorption characteristics which result in longer lasting concentrations of KY-109 in lesions.

The potent broad-spectrum activities of KY-109 make this compound a potentially useful therapeutic agent.

References

1) KAKEYA, N.; S. NISHIZAWA, K. NISHIMURA, A. YOSHIMI, S. TAMAKI, T. MORI & K. KITAO: KY-109, a new bifunctional pro-drug of a cephalosporin. Chemistry, physico-chemical and biological properties. J. Antibiotics 38: 380~389, 1985
2) LITCHFIELD, J. T. & F. WILCOXON: A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96: 99~113, 1949
3) SAITO, M.; T. SAITO, K. YANO, K. SHIBATA, T. NISHINO & T. TANINO: Therapeutic effect of cefotetan in an experimental local infection with facultative and obligate anaerobes in granuloma pouches in rats. J. Antimicrob. Chemother. 13: 133~142, 1984
4) SAKAMOTO, N.: An experimental chemotherapy on pyelonephritis in rabbits inoculated with *Escherichia coli*. Chemotherapy (Tokyo) 28: 31~48, 1980