IN VITRO AND *IN VIVO* ANTIBACTERIAL ACTIVITY OF KT3777, A NEW ORALLY ACTIVE CARBACEPHEM

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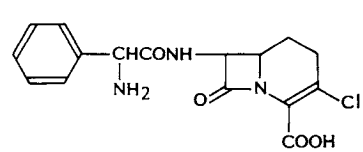
KT3777 is a novel carbacephem antibiotic structurally identical to cefaclor (CCL), except that the sulfur atom of position 1 of the cephem nucleus has been replaced by carbon. KT3777 was investigated for *in vitro* and *in vivo* antibacterial activities in comparison with CCL, cephalexin (CEX) and amoxicillin. The MIC_{50} of KT3777 ranged from 0.2 to 3.13 μg/ml for clinical isolates of *Staphylococcus aureus*, Streptococci, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Haemophilus influenzae*, and *Neisseria gonorrhoeae*. KT3777 possessed an antibacterial spectrum and potency similar to that of CCL. However, against *E. coli* and *K. pneumoniae*, KT3777 was about twice as active as CCL. KT3777 was more active than CEX against all strains tested. Killing-curve studies demonstrated bactericidal activity of KT3777 at concentrations above the MIC. KT3777 showed good affinity for penicillin-binding proteins 1A, 1Bs, 3 and 4 of *E. coli* NIHJ JC-2. The protective effect of KT3777 against systemic infections in mice was comparable to that of CCL with a few exceptions and about 3 to 7 times greater than that of CEX. KT3777 also proved effective against localized infections such as acute pneumonia and ascending urinary tract infections in mice.

There has been continued interest in the development of oral β-lactam antibiotics that can be used to treat infections caused by Gram-positive and Gram-negative bacteria[1~3]. KT3777 (LY163892, Eli Lilly) is a novel oral β-lactam antibiotic in the carbacephem class. That was synthesized by Kyowa Hakko Kogyo Co., Ltd.[4~6]. Its chemical name is 7-[D-(aminophenylacetyl)amino]-3-chloro-8-oxo-1-azabicyclo-[4,2,0]oct-2-ene-2-carboxylic acid. Chemical structure is shown in Fig. 1. Its structure is similar to that of cefaclor (CCL), the only difference being substitution of carbon for sulfur at the number 1 position in the dihydrothiazine ring. KT3777 possesses an antibacterial spectrum virtually identical to that of CCL[7,8]. KT3777 resists hydrolysis by plasmid-mediated β-lactamase[9]. Previous experience in our laboratory indicated that KT3777 has greater bioavailability superior to that of CCL after oral administration in experimental animal such as dogs and monkeys, and is more chemically stable than CCL[10].

In this paper, we compared the *in vitro* and *in vivo* antibacterial activity of KT3777 with those of CCL, cephalexin (CEX) and amoxicillin. This work was presented in part at the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy[10].

Fig. 1. Chemical structure of KT3777.



Materials and Methods

Antibiotics

KT3777 was prepared at Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan. CCL and CEX (Shionogi & Co., Ltd., Osaka, Japan) and amoxicillin (Kyowa Hakko Kogyo Co., Ltd.) were obtained from commercial sources.

Organisms

Stock strains from the culture collection in our laboratories were used in this study. Clinical isolates of various species of bacteria were obtained from several hospitals in Japan. Anaerobes were kindly provided by Dr. K. UENO, Institute of Anaerobic Bacteriology, Gifu University School of Medicine.

Determination of MIC

The MIC of the test antibiotics were determined by the agar dilution method. Mueller-Hinton agar (Difco Laboratories, U.S.A.) was used for nonfastidious aerobic bacteria. Mueller-Hinton agar supplemented with 5% defibrinated horse blood was used for *Streptococcus* species and *Enterococcus* species. Mueller-Hinton agar supplemented with 5% defibrinated horse blood and heated (chocolate agar) was used for *Haemophilus influenzae*. Thayer-Martin agar (Nissui, Japan) containing 1% hemoglobin and GAM agar (Nissui) were used for *Neisseria gonorrhoeae* and anaerobic bacteria, respectively. All antibiotics solutions were freshly made on the day of use. All strains except *Streptococcus* species, *Enterococcus* species, *H. influenzae*, *N. gonorrhoeae* and anaerobic bacteria were grown overnight in Mueller-Hinton broth (Difco). *Streptococcus* species, *Enterococcus* species and *H. influenzae* were grown overnight in Mueller-Hinton broth plus 5% defibrinated horse blood. *N. gonorrhoeae* was grown overnight on Thayer-Martin agar containing 1% hemoglobin. GAM broth was used for anaerobic bacteria. The overnight broth cultures and bacterial suspensions of *N. gonorrhoeae* were diluted to a final concentration of approximately 10^6 cfu/ml, and one loopful of each bacterial suspension, corresponding to about 10^4 cfu, was inoculated (Microplanter; Sakuma Seisakusho, Japan) onto agar plates that contained 2-fold serial dilutions of antibiotics. The plates were incubated for 18 hours at 37°C except the methicillin-resistant *Staphylococcus aureus* and *N. gonorrhoeae* plates, which were incubated for 18 hours at 30°C and for 48 hours at 37°C, respectively. *H. influenzae*, *N. gonorrhoeae* and anaerobic bacteria were incubated in the GasPak system (BBL Microbiology Systems, U.S.A.) at 37°C. The MIC was defined as the lowest concentration of the antibiotic that prevented visible growth. The MIC_{50} and MIC_{90} were the concentrations of a drug required to inhibit 50% and 90% of strains, respectively.

Bactericidal Activity

The bactericidal activity of KT3777 was measured against *Escherichia coli* F3385 and *Klebsiella pneumoniae* F1928 in Mueller-Hinton broth. An overnight culture was suspended in fresh medium at about 10^4 cfu/ml and incubated at 37°C with shaking. After 2 hours of incubation, the test antibiotic was added to the culture at various concentrations around the MIC. At intervals during incubation for 6 hours at 37°C, samples were removed, immediately diluted in broth, and plated on Mueller-Hinton agar. The numbers of colonies were counted after 18 hours of incubation at 37°C.

Affinity for Penicillin-binding Proteins (PBPs)

The affinity of KT3777 for PBPs was examined by a competition method described previously[11,12]. A total membrane fraction was prepared from *E. coli* NIHJ JC-2 exponentially grown in Antibiotic medium No. 3 (Difco), and suspended at 20 mg/ml in 0.05 M sodium phosphate buffer. 100 μl of the membrane preparation was preincubated with 5 μl of either 0.05 M phosphate buffer or a dilution of nonradioactive antibiotic for 10 minutes at 30°C. 10 μl of [^{14}C]benzylpenicillin (specific activity; 54 mCi/mmol, Amersham International plc, England) was added and incubated for a further 10 minutes at 30°C. The reaction was terminated by the addition of unlabeled benzylpenicillin and sarkosyl. After the suspension was allowed to stand for 20 minutes at 25°C, the inner membrane fraction (Sarkosyl soluble fraction) was obtained by centrifugation at 15,000 $\times g$ for 30 minutes at 25°C. The fraction was mixed with 50 μl of 0.5 M Tris-HCl buffer (pH 6.8) containing sodium dodecyl sulfate 3% (w/v), glycerol 30%

(w/v), bromphenol blue 0.002% (w/v), and 10 μl of 2-mercaptoethanol, and heated in a boiling water bath for 3 minutes. 50 μl of the solution was then subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. After electrophoresis, the gel was fixed in 300 ml of 50% methanol - 7% acetic acid for 1 hour at room temperature and washed 5 times for 0.5 hour with 300 ml of 5% methanol - 7% acetic acid. The gel was soaked in 300 ml of 20% (w/v) 2,5-diphenyloxazole (PPO) in dimethyl sulfoxide with gentle shaking for 3 hours, washed in water, and dried *in vacuo* on Whatman No. 3 MM paper. A fluorogram was prepared by exposing the gel to X-ray film (Ultrofilm ^{3}H, LKB) at −80°C for 3 months. The concentration of antibiotics required for 50% binding inhibition of ^{14}C-labeled benzylpenicillin to PBPs (IC_{50}s) were determined with a densitometer (Model CS-900, Shimadzu, Japan).

Experimental Infections in Mice

Systemic Infections: Ten 4-week-old male *dd*Y strain mice weighing 18 to 20 g were used for each dose level. Test organisms were cultured in Trypto - Soya broth (TSB, Nissui, for *Streptococcus* species) or in Brain Heart Infusion broth (BHIB, Difco, for other organism) for 18 hours at 37°C. Subcultures, which were incubated for 4 hours at 37°C after being suitably diluted with fresh TSB or BHIB, were suspended in the same amount of physiological saline (*Streptococcus pneumoniae* Type III) or 10% gastric mucin (Difco). A 0.5-ml volume of the bacterial suspension was injected intraperitoneally. The challenge doses ranged from 2 to 10 times the minimal lethal doses. Under these conditions, all untreated mice died within 48 hours. The drugs were suspended in 0.3% carboxymethylcellulose and administered orally at 2 hours after infection, except *E. coli* GN2411-5 (1, 3 and 5 hours). The 50% effective dose (ED_{50}) was calculated by the LITCHFIELD-WILCOXON method[13] according to the numbers of survivors at 7 days after infection.

Respiratory Tract Infection: Acute bronchopneumonia in mice caused by *K. pneumoniae* F3740 was induced at described previously[14]. Male ICR mice weighing 18 to 20 g were placed in an exposure chamber (Okazaki Sangyo, Tokyo, Japan). A 10-ml of bacterial suspension of *K. pneumoniae* F3740 (4.1×10^9 cfu/ml), charged in a nebulizer (NE-11B, OMRON, Japan) was aerosolized with compressed air for 30 minutes. This resulted in a deposition of about 10^4 cfu per lung, and no mice died within 3 days after infection. The antibiotics were administered orally to groups of five mice at 18, 21 and 24 hours after infection. At 20 hours after the last medication, lungs were removed aseptically, homogenized in 2 ml of saline and viable bacterial cells were counted by conventional plating techniques.

Ascending Urinary Tract Infection: A modified method described by OOMORI *et al.*[15] for experimental urinary tract infection in mice was used. Female 4-week-old mice weighing 18 to 20 g were used. After restriction of water intake for 24 hours, mice were challenged by transurethral inoculation with 50 μl of an *E. coli* GN2411-5 suspension (2.1×10^6 cfu/ml). Immediately afterwards, the external urethral meatus was clamped for 1 hour. Drug were administered orally to groups of five mice at 1, 3 and 5 hours after infection. At 24 hours after infection, kidney were removed aseptically and homogenized, and viable bacterial cells were counted by conventional plating techniques. In all of the untreated mice, the procedure gave rise to kidney infections with histopathological feature of pyelonephritis within 24 hours.

Results

Antibacterial Spectrum and Susceptibility of Clinical Isolates

To assess the antibacterial spectrum of KT3777, its antibacterial activity against typical laboratory strains was compared with that of CCL, CEX and amoxicillin (Table 1). The MICs of KT3777 ranged from 0.2 to 1.56 μg/ml for *Staphylococcus* species, *Streptococcus* species, *Micrococcus luteus*, *Bacillus subtilis*, *E. coli*, *K. pneumoniae*, *Proteus mirabilis* and *H. influenzae*. KT3777 was inactive against methicillin-resistant *S. aureus* (MRSA), *Proteus vulgaris*, *Morganella morganii*, *Enterobacter* species, *Serratia marcescens* and *Pseudomonas aeruginosa*. The antibacterial spectrum and potency of KT3777 against aerobes was considered to be similar to that of CCL but slightly superior to that of CEX. KT3777 inhibited some species of Gram-positive anaerobes, such as *Peptostreptococcus*, *Propionibacterum* and *Eubacterium* but showed no

Table 1. Antibacterial spectrum of KT3777 and reference antibiotics against aerobes.

Organism	MIC (μg/ml)			
	KT3777	CCL	CEX	Amoxicillin
Staphylococcus aureus FDA 209P JC-1	0.2	0.2	0.78	0.05
S. aureus Smith	0.78	0.78	1.56	0.2
S. aureus J555[a]	>100	>100	>100	100
S. epidermidis	1.56	1.56	3.13	0.2
Streptococcus pyogenes S 23	0.2	0.1	0.39	0.01
S. pyogenes Cook	0.2	0.1	0.39	0.01
S. pneumoniae Type 1	0.39	0.39	1.56	0.01
S. pneumoniae Type 2	0.78	0.39	3.13	0.01
S. pneumoniae Type 3	1.56	0.78	6.25	0.01
Enterococcus faecalis ATCC 10541	25	25	>100	0.2
Micrococcus luteus ATCC 9341	0.39	0.2	1.56	0.2
Bacillus subtilis ATCC 6633	0.2	0.1	0.78	0.02
Escherichia coli NIHJ JC-2	0.78	0.78	3.13	3.13
E. coli GN2411-5	0.39	0.78	3.13	1.56
Klebsiella pneumoniae F3800	0.39	0.78	3.13	50
Proteus mirabilis 1287	1.56	1.56	12.5	0.78
P. vulgaris 6897	>100	25	25	50
P. inconstans F3301	6.25	50	25	>100
Providencia rettgeri 4289	50	>100	>100	25
Morganella morganii KY4298	100	>100	>100	>100
Enterobacter cloacae F1870	>100	>100	>100	>100
E. aerogenes F1949	>100	>100	>100	>100
Citrobacter freundii F1905	12.5	25	50	>100
Serratia marcescens T-26	100	>100	>100	50
Pseudomonas aeruginosa No. 1	>100	>100	>100	>100
Haemophilus influenzae F4270	1.56	1.56	6.25	0.2

Agar dilution method, inoculum size 10^6 cfu/ml.
[a] Methicillin-resistant.

Table 2. Antibacterial spectrum of KT3777 and reference antibiotics against selected anaerobes.

Organism	MIC (μg/ml)			
	KT3777	CCL	CEX	Amoxicillin
Peptostreptococcus prevotii 1911	0.2	0.39	0.39	0.02
P. asaccharolyticus 0665	0.78	0.78	3.13	0.2
P. asaccharolyticus 1684	0.1	0.2	0.2	0.02
P. magnus 1694	0.78	1.56	3.13	0.2
P. magnus 1908	1.56	1.56	6.25	0.2
P. variabilis 0670	1.56	1.56	6.25	0.2
P. anaerobius 1705	0.78	1.56	3.13	0.1
Propionibacterium acnes 1827	1.56	1.56	6.25	0.2
Eubacterium lentum 0309	6.25	12.5	6.25	0.78
Bifidobacterium adolescentis 0364	100	>100	50	25
Clostridium difficile 0745	100	>100	50	25
Veillonella parvula 2046	>100	>100	50	25
Bacteroides fragilis 0004	100	>100	50	25
B. fragilis 7000	100	>100	50	25
B. thetaiotaomicron 0659	100	>100	50	25
Fusobacterium mortiferum 0304	100	50	25	1.56

Agar dilution method, inoculum size 10^6 cfu/ml.

Table 3. *In vitro* antibacterial activities of KT3777 and reference antibiotics against various clinical isolates.

Organism (No.)	Antibiotics	MIC (μg/ml)[a]		
		Range	50%	90%
Staphylococcus aureus (51)	KT3777	0.39～>100	1.56	100
	CCL	0.39～>100	1.56	100
	CEX	0.78～>100	3.13	100
	Amoxicillin	0.1 ～ 100	1.56	50
S. aureus	KT3777	>100	>100	>100
(methicillin-resistant) (27)	CCL	>100	>100	>100
	CEX	100～>100	>100	>100
	Amoxicillin	25～>100	100	>100
S. epidermidis (15)	KT3777	0.78～50	6.25	12.5
	CCL	0.78～25	6.25	12.5
	CEX	1.56～50	12.5	25
	Amoxicillin	0.39～50	1.56	6.25
Streptococcus pyogenes (27)	KT3777	0.1～0.39	0.2	0.2
	CCL	0.1～0.2	0.2	0.2
	CEX	0.2～0.78	0.78	0.78
	Amoxicillin	0.01～0.01	0.01	0.02
S. pneumoniae (11)	KT3777	0.39～1.56	0.78	1.56
	CCL	0.39～0.78	0.39	0.39
	CEX	0.78～6.25	1.56	3.13
	Amoxicillin	0.01～0.01	0.01	0.01
Enterococcus faecalis (18)	KT3777	100～>100	>100	>100
	CCL	50～100	50	100
	CEX	100～>100	>100	>100
	Amoxicillin	0.39～0.78	0.39	0.78
E. faecium (6)	KT3777	100～>100	>100	>100
	CCL	50～>100	100	>100
	CEX	>100	>100	>100
	Amoxicillin	0.39～25	0.78	25
Escherichia coli (54)	KT3777	0.2～>100	0.78	25
	CCL	0.39～>100	1.56	50
	CEX	1.56～>100	6.25	>100
	Amoxicillin	1.56～>100	50	>100
Klebsiella pneumoniae (54)	KT3777	0.2～>100	0.39	1.56
	CCL	0.2～>100	0.78	3.13
	CEX	1.56～>100	3.13	12.5
	Amoxicillin	12.5～>100	100	>100
Proteus mirabilis (50)	KT3777	0.39～12.5	0.78	1.56
	CCL	0.39～12.5	1.56	1.56
	CEX	6.25～50	12.5	25
	Amoxicillin	0.39～>100	0.78	1.56
P. inconstans (27)	KT3777	0.78～>100	12.5	100
	CCL	3.13～>100	25	>100
	CEX	6.25～>100	25	>100
	Amoxicillin	3.13～>100	100	>100
Citrobacter freundii (27)	KT3777	6.25～>100	>100	>100
	CCL	12.5～>100	>100	>100
	CEX	25～>100	>100	>100
	Amoxicillin	>100～>100	>100	>100
Enterobacter cloacae (27)	KT3777	0.78～>100	50	>100
	CCL	0.39～>100	100	>100
	CEX	3.13～>100	>100	>100
	Amoxicillin	6.25～>100	>100	>100

Table 3. (Continued)

Organism (No.)	Antibiotics	MIC (μg/ml)[a]		
		Range	50%	90%
Serratia marcescens (15)	KT3777	>100	>100	>100
	CCL	>100	>100	>100
	CEX	>100	>100	>100
	Amoxicillin	>100	>100	>100
Haemophilus influenzae (11)	KT3777	0.78~1.56	1.56	1.56
	CCL	0.78~3.13	1.56	1.56
	CEX	6.25~25	6.25	12.5
	Amoxicillin	0.2~0.39	0.2	0.39
Neisseria gonorrhoeae (22)	KT3777	0.78~3.13	1.56	1.56
	CCL	0.78~3.13	1.56	3.13
	CEX	12.5~50	12.5	25
	Amoxicillin	0.39~1.56	0.78	1.56
N. gonorrhoeae (penicillinase-producing strains) (15)	KT3777	0.39~25	3.13	3.13
	CCL	0.2~25	1.56	12.5
	CEX	0.78~25	12.5	25
	Amoxicillin	25~>100	>100	>100
Bacteroides fragilis (20)	KT3777	50~>100	100	>100
	CCL	50~>100	>100	>100
	CEX	25~>100	50	>100
	Amoxicillin	12.5~>100	20	>100

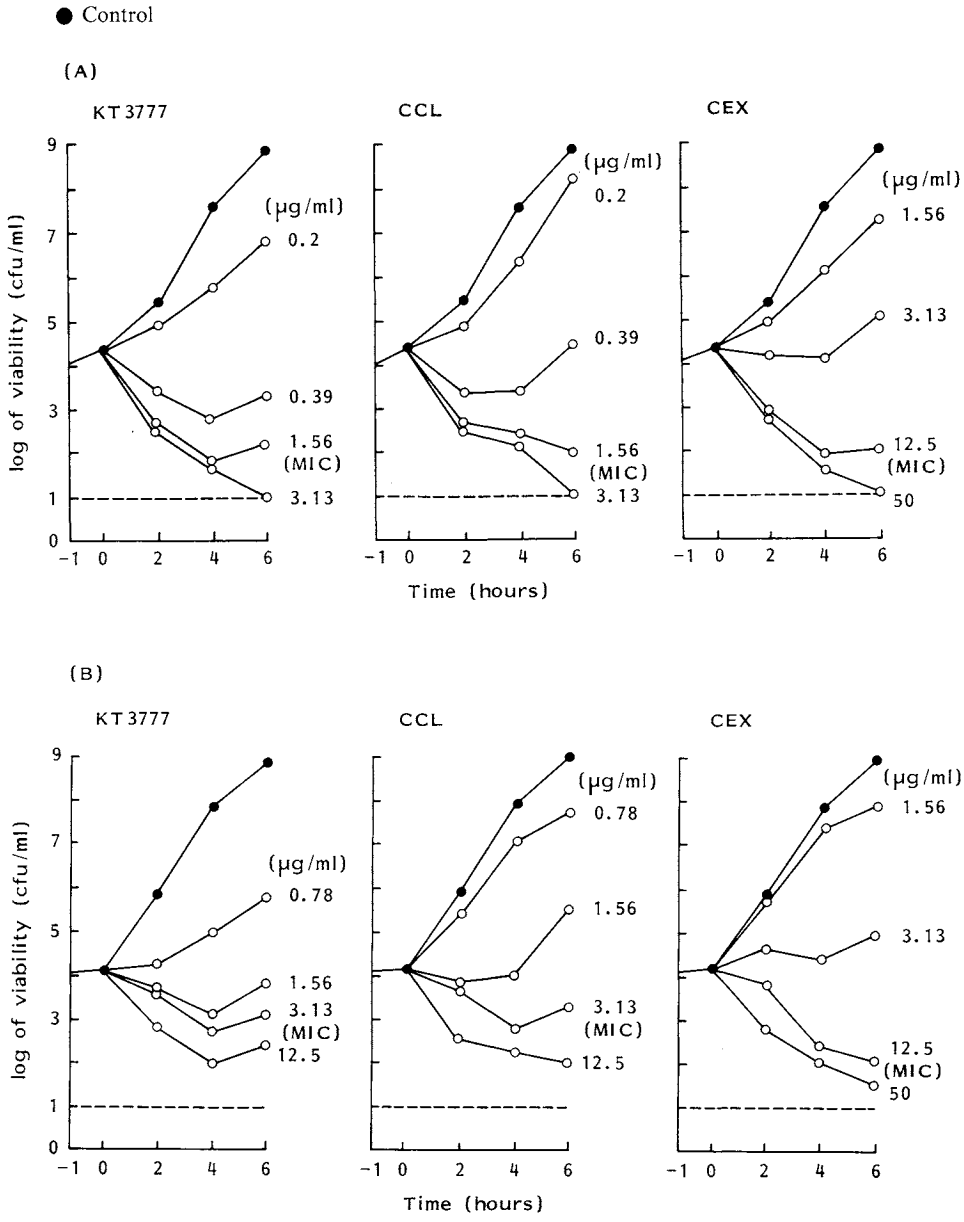
[a] Agar dilution method; inoculum size 10^6 cfu/ml; 50 and 90%, MIC of the antibiotic that inhibited 50 and 90%, respectively, of the isolates.

activity against Gram-negative anaerobes (Table 2). The susceptibility of clinical isolates to KT3777 and the reference antibiotics is shown in Table 3. The lowest concentration of KT3777 at which 50% of the clinical isolates were inhibited (MIC_{50}) ranged from 0.2 to 3.13 μg/ml for *S. aureus*, Streptococci, *E. coli*, *K. pneumoniae*, *P. mirabilis*, *H. influenzae* and *N. gonorrhoeae* (including penicillinase-producing strains). For *Staphylococcus epidermidis*, *Proteus inconstans* and *Enterobacter cloacae*, the MIC_{50} of KT3777 ranged from 6.25 to 50 μg/ml. KT3777 showed little or no antibacterial activity against methicillin-resistant *S. aureus* (MRSA), Enterococci, *Citrobacter freundii*, *S. marcescens* and *Bacteroides fragilis*. The activity of KT3777 was comparable to that of CCL against Staphylococci, Streptococci, *H. influenzae* and *N. gonorrhoeae*. KT3777 showed slightly higher activity than CCL against several members of the Enterobacteriaceae, such as *E. coli* and *K. pneumoniae*. CEX was 2 to 16 times inferior to KT3777 in activity against all Gram-positive and Gram-negative bacteria tested, in particular *E. coli*, *K. pneumoniae*, *P. mirabilis* and *N. gonorrhoeae*. The activity of amoxicillin against Streptococci, Enterococci and *H. influenzae* was much better than that of KT3777 and the other antibiotics, whereas amoxicillin displayed weaker activity than KT3777 against *E. coli* and *K. pneumoniae*.

Bactericidal Activity

Time-kill curves of KT3777 are shown in Fig. 2. The bactericidal activity of KT3777 was compared with that of CCL and CEX against *E. coli* F3385 and *K. pneumoniae* F1928. Rapid reduction of cfu in *E. coli* F3385 was found with the addition of drugs at concentrations above the MIC (MICs: KT3777 and CCL, 1.56 μg/ml; CEX, 12.5 μg/ml). Killing of more than 99% of the initial viable cells was achieved within 2 hours of incubation after exposure to these antibiotics at concentrations higher than the MIC. Moreover, no regrowth was observed during 24 hours of incubation at concentrations above the MIC.

Fig. 2. Bactericidal activity of KT3777, CCL and CEX against *Escherichia coli* F3385 (A), and *Klebsiella pneumoniae* F1928 (B).

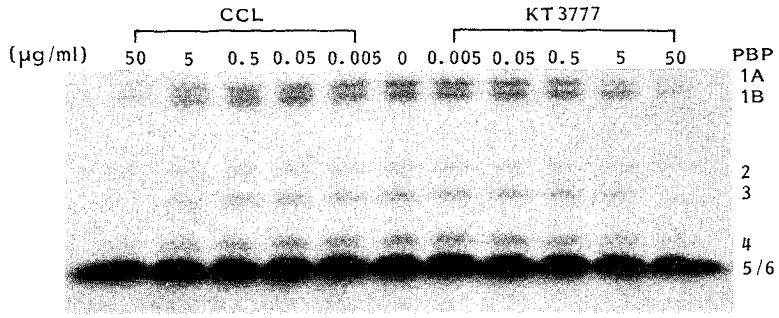


KT3777 and the reference drugs exhibited bactericidal activity against *K. pneumoniae* at concentrations above the MIC (MICs: KT3777 and CCL, 3.13 μg/ml; CEX, 12.5 μg/ml). Reductions in viability greater than 99% were seen with the addition of 4-fold the MIC of each drug within 4 to 6 hours. No regrowth occurred after exposure to 4-fold the MIC of each drug up to 24 hours.

Binding Affinity for PBPs

The binding affinity of KT3777 for PBPs was compared with that of CCL. The results of fluorography by competition of KT3777 with ^{14}C-labeled benzylpenicillin for binding to PBPs of *E. coli* NIHJ JC-2

Fig. 3. Fluorography by competition of KT3777 and CCL with [^{14}C]benzylpenicillin for PBPs of *Escherichia coli* NIHJ JC-2.



MIC (μg/ml): KT3777 0.78, CCL 0.78.

Table 4. Protective effect of KT3777, CCL and CEX against systemic infections in mice.

Organism	Challenge cells (cfu/mouse)	Antibiotic	ED_{50} (mg/kg) (95% confidence limits)	MIC (μg/ml)
Staphylococcus aureus Smith		KT3777	0.19 (0.11~0.32)	0.78
	2.2×10^5	CCL	0.25 (0.17~0.34)	0.78
		CEX	0.53 (0.30~0.91)	1.56
Streptococcus pyogenes S 23		KT3777	0.41 (0.25~0.69)	0.2
	3.3×10^5	CCL	0.27 (0.20~0.36)	0.1
		CEX	1.46 (1.06~2.03)	0.39
S. pneumoniae Type 3		KT3777	21.0 (12.3~35.9)	1.56
	1.2×10^2	CCL	6.5 (4.4~9.5)	0.78
		CEX	34.2 (26.1~44.9)	6.25
Escherichia coli GN2411-5		KT3777	4.6 (3.4~6.4)	0.39
	1.2×10^3	CCL	5.3 (3.8~7.3)	0.78
		CEX	33.2 (17.2~63.9)	3.13
E. coli F1095		KT3777	10.8 (7.1~16.4)	0.78
	1.0×10^3	CCL	13.3 (7.9~22.2)	1.56
		CEX	59.7 (36.7~97.1)	6.25
Klebsiella pneumoniae 8045	1.2×10^4	KT3777	2.9 (2.0~4.1)	0.39
		CCL	2.4 (1.8~3.3)	0.39
Proteus mirabilis 1287	1.4×10^6	KT3777	83.7 (55.1~127.0)	1.56
		CCL	15.3 (10.2~23.0)	1.56

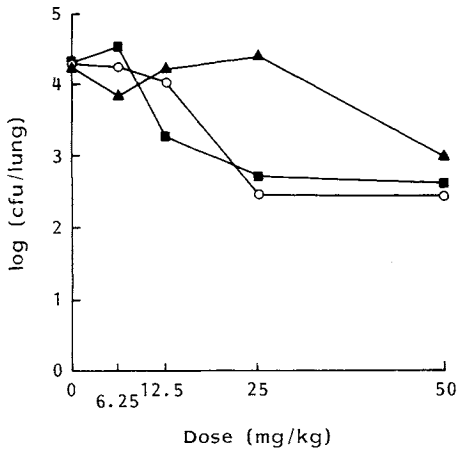
Mouse: *dd* Y, ♂, 18~20 g, $n=10$. Challenge: ip. Therapy: po.
ED_{50} was determined by LITCHFIELD-WILCOXON method based on mice surviving 7 days after challenge.

are shown in Fig. 3. KT3777 possessed moderate affinities for PBP1A (IC_{50}, 2.7 μg/ml), PBP3 (IC_{50}, 3.6 μg/ml) and PBP4 (IC_{50}, 2.6 μg/ml), and relatively low affinity for PBP1B (IC_{50}, 18.6 μg/ml). CCL also had moderate affinities for PBP1A (IC_{50}, 5.9 μg/ml), PBP1B (IC_{50}, 9.8 μg/ml), PBP3 (IC_{50}, 2.5 μg/ml) and PBP4 (IC_{50}, 7.2 μg/ml). KT3777 showed 2 to 3 times higher affinities for PBP1A and PBP4 than did CCL, whereas it had about 2 times lower affinity for PBP1B. Both KT3777 and CCL showed only poor affinities for PBP2, PBP5 and PBP6.

Therapeutic Effects *In Vivo*

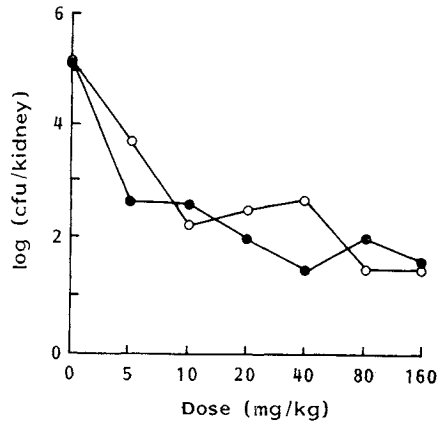
The protective effects of KT3777 and the reference drugs against systemic infections in mice caused by a variety of pathogens are summarized in Table 4. Against the mice infected with *S. aureus* Smith, *S. pyogenes* S 23, *E. coli* GN2411-5, *E. coli* F1095 and *K. pneumoniae* 8045, the protective effect of KT3777

Fig. 4. Therapeutic efficacy of KT3777 (○), CCL (■) and CEX (▲) against experimental respiratory tract infection in mice.



The experiment was done as described in the text. MIC (μg/ml): KT3777 0.39, CCL 0.39, CEX 3.13.

Fig. 5. Therapeutic efficacy of KT3777 (●) and CCL (○) against experimental urinary tract infection in mice.



The experiment was done as described in the text. MIC (μg/ml): KT3777 0.39, CCL 0.78.

was comparable to that of CCL, and about 3 to 7 times greater than that of CEX. KT3777 was 3 to 5 times inferior to CCL against *S. pneumoniae* and *P. mirabilis* infections. Against *S. pneumoniae* infection, KT3777 was slightly more active than CEX. The therapeutic efficacy of KT3777 against acute pneumonia induced by *K. pneumoniae* F3740 in mice is shown in Fig. 4. KT3777 was as effective as CCL and more effective than CEX in reducing the number of viable bacteria in the lungs. At a dose of 25 mg/kg, the viable cell counts (log cfu/lung) were 2.40 ± 1.37 for KT3777, 2.67 ± 0.57 for CCL, 4.33 ± 0.62 for CEX, whereas they were 4.27 ± 0.72 for the untreated control. The therapeutic effect of KT3777 against an ascending urinary tract infection in mice caused by the transurethral inoculation of *E. coli* GN2411-5 was compared with that of CCL (Fig. 5). Bacterial numbers increased to about 5.14 cfu/kidney (log) in the untreated control. In contrast, treatment with oral doses of KT3777 led to a two log reduction in the numbers of viable cells. KT3777 was comparable to CCL in reducing the numbers of viable bacteria in the kidney.

Discussion

In this report, the *in vitro* and *in vivo* antibacterial activities of KT3777 were compared with CCL[16,17], CEX and amoxicillin, which were commercially available oral β-lactam antibiotics.

KT3777 exhibited good antibacterial activity against *S. aureus*, Streptococci, *E. coli*, *K. pneumoniae*, *P. mirabilis*, *H. influenzae* and *N. gonorrhoeae*. Its activity was comparable to that of CCL and higher than that of CEX. However, KT3777 showed higher activity than CCL against several members of the Enterobacteriaceae, such as *E. coli* and *K. pneumoniae*, suggesting an advantage for these species. KT3777 also inhibited Gram-positive anaerobes, such as *Peptostreptococcus*, *Propionibacterium* and *Eubacterium*. Like first generation cephalosporins, KT3777 showed poor or no antibacterial activity against methicillin-resistant *S. aureus*, Enterococci, *C. freundii*, *S. marcescens*, *P. aeruginosa* and *B. fragilis*. KT3777 showed bactericidal activity at MIC concentrations or greater against *E. coli* and *K. pneumoniae*. This activity was similar to that of CCL and slightly superior to that of CEX. KT3777 is more stable to plasmid-mediated β-lactamases than CCL[7~9]. In our studies, KT3777 had a β-lactamase stability slightly superior to or similar to that of CCL against various β-lactamase producing clinical isolates (data not shown). KT3777 possessed high affinities for PBP1A, PBP3 and PBP4 of *E. coli* NIHJ JC-2, and moderate

affinity for PBP1B. KT3777 also showed less affinities for PBP2, PBP5 and PBP6. KT3777 showed 2 to 3 times higher affinities for PBP1A and PBP4 than CCL, whereas it was about 2 times lower affinity for PBP1B than CCL.

Against systemic infections, the protective effect of KT3777 after oral dosing was almost comparable to that of CCL, with the exception of *S. pneumoniae* and *P. mirabilis* infections. KT3777 was uniformly more effective than CEX against bacterial systemic infections tested. Furthermore against localized infections such as acute pneumonia and ascending pyelonephritis in mice, KT3777 was as effective as CCL in reducing the numbers of viable bacteria in the target organ (lung or kidney).

We have confirmed two advantages in our previous experiments. That is, KT3777 has greater bioavailability and greater chemical stability than CCL (under preparation).

In conclusion, KT3777 is the first carbacephem antibiotic for oral use. These results of laboratory studies suggest that this compound would be useful in the clinical treatment of bacterial infections. Recently, clinical efficacy studies of KT3777 have been started.

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