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In Vitro Antibacterial Activity of KP-736, a New Cephem Antibiotic

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KP-736, a new cephem antibiotic with a broad antibacterial spectrum and potent antipseudomonal activity, was evaluated for in vitro antibacterial activity in comparison with ceftazidime, cefotaxime, and cefpirome. KP-736 was significantly more active than the test compounds against gram-negative bacteria, including the *Pseudomonas* group and ceftazidime-, cefotaxime-, or imipenem-resistant strains, but less active against gram-positive bacteria. KP-736 had very high affinities for penicillin-binding protein 3 (PBP 3) of *Escherichia coli* K-12 and PBP 1A and PBP 3 of *Pseudomonas aeruginosa* NCTC 10490 and showed potent bactericidal activities against these two strains. It was stable to hydrolysis by penicillinases and cephalosporinases but was slightly hydrolyzed by oxyiminocephalosporinases and type II penicillinase.

A number of β-lactam antibiotics with a broad antibacterial spectrum of activity have been developed during the past decade. However, the clinical effectiveness of these drugs has been limited owing to the rapid development of resistance of *Pseudomonas aeruginosa* and certain members of the family *Enterobacteriaceae*, such as *Citrobacter* and *Enterobacter* spp., in immunocompromised patients (17–19). Recently, several β-lactam antibiotics containing catechol derivatives in the structure have been reported to possess excellent antibacterial activity against *P. aeruginosa* (1, 3, 6, 10, 11, 13, 14, 16).

KP-736, disodium (6*R*,7*R*)-7-[(*z*)-2-(2-aminothiazole-4-yl)-2-(1,5-dihydroxy-4-pyridon-2-ylmethoxyimino)acetamido]-3-(1,2,3-thiadiazole-5-ylthiomethyl)-3-cephem-4-carboxylate (Fig. 1), is a new cephem with a broad antibacterial spectrum and potent antipseudomonal activity, synthesized at Kaken Pharmaceutical Co., Ltd., Tokyo, Japan. In this paper, we report the in vitro activity of KP-736 in comparison with that of ceftazidime (24), cefotaxime (8), and cefpirome (5) against gram-positive and gram-negative clinical isolates including strains resistant to ceftazidime, cefotaxime, or imipenem. In addition, binding affinity for the target, penicillin-binding proteins (PBPs), and stability against hydrolysis by β-lactamases are presented.

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MATERIALS AND METHODS

Antibiotics. KP-736 was synthesized at the Central Research Laboratories, Kaken Pharmaceutical Co., Ltd. Other compounds were obtained as follows: ceftazidime and cephaloridine, Nihon Glaxo, Co., Tokyo, Japan; cefotaxime and cefpirome, Hoechst Japan, Co., Tokyo, Japan; cefoxitin, Daiichi Pharmaceutical Co., Tokyo, Japan; imipenem, Banyu Pharmaceutical Co., Tokyo, Japan; and penicillin G, Meiji Seika Kaisha, Ltd., Tokyo, Japan.

Organisms. Bacterial strains used in this study were recent clinical isolates collected from various laboratories and hospitals in Japan. All isolates were maintained as stock cultures at the Episome Institute. Imipenem-resistant *P. aeruginosa* strains were collected independently of the other strains.

Determination of MICs. MICs were determined by the twofold agar dilution method with sensitivity disk agar-N (SDA-N; Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), which is modified Mueller-Hinton agar, unless otherwise specified, as previously described (7). The overnight broth cultures of the bacterial strains were diluted with corresponding broth or buffered saline containing 0.01% gelatin to a final concentration of about 5×10^6 CFU/ml. Then 5 μl of each bacterial suspension, corresponding to about 10^4 CFU, was spotted with an inoculator (Microplanter; Sakuma Seisakusho, Tokyo, Japan) onto an agar plate containing twofold serial dilutions of antibiotics. The inoculated plates were incubated for 18 h at 37°C, except for those containing *Haemophilus influenzae* and *Neisseria gonorrhoeae*, which were incubated in a candle extinction jar for 18 h, and those containing anaerobes, which were incubated in an anaerobic chamber for 24 h. The MIC was defined as the lowest concentration of the compound that prevented visible growth on the agar plate. MICs were also determined by the twofold broth dilution method. Each 1-ml portion containing a serial dilution of the compounds in sensitivity test broth (STB; Nissui) was inoculated with overnight cultures in STB at 37°C to yield a final inoculum about 10^6 CFU/ml. The tubes were incubated for 18 h at 37°C. The MIC was defined as the lowest concentration of the compound that prevented visible growth in broth.

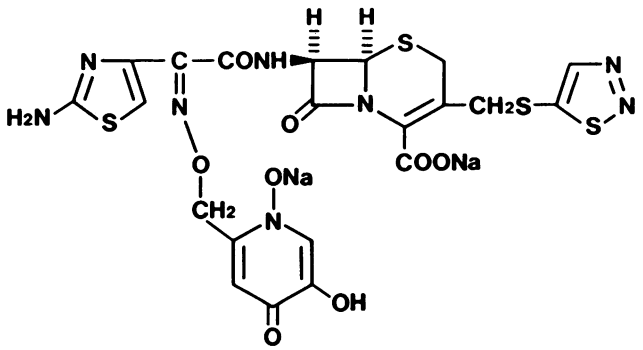

FIG. 1. Structure of KP-736.

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TABLE 1. Activities of KP-736 against clinical isolates

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus aureus, methicillin susceptible (54)	KP-736	6.25–25	25	25
	Ceftazidime	3.13–12.5	12.5	12.5
	Cefotaxime	1.56–6.25	3.13	3.13
	Cefpirome	0.39–1.56	0.78	0.78
Staphylococcus aureus, methicillin resistant (53)	KP-736	50–>100	>100	>100
	Ceftazidime	12.5–>100	100	>100
	Cefotaxime	12.5–>100	>100	>100
	Cefpirome	3.13–100	50	100
Staphylococcus epidermidis (79)	KP-736	12.5–>100	50	>100
	Ceftazidime	3.13–>100	12.5	>100
	Cefotaxime	0.10–>100	6.25	>100
	Cefpirome	0.10–50	0.78	6.25
Streptococcus pneumoniae (24)	KP-736	0.20–0.39	0.20	0.39
	Ceftazidime	0.10–0.39	0.20	0.39
	Cefotaxime	0.013–0.025	0.013	0.025
	Cefpirome	0.013–0.05	0.025	0.025
Streptococcus pyogenes (92)	KP-736	0.20–0.78	0.39	0.39
	Ceftazidime	0.10–0.39	0.10	0.20
	Cefotaxime	≤0.006–0.025	0.013	0.013
	Cefpirome	≤0.006–0.025	0.013	0.013
Enterococcus faecalis (99)	KP-736	12.5–>100	50	>100
	Ceftazidime	12.5–>100	100	>100
	Cefotaxime	0.39–>100	12.5	>100
	Cefpirome	1.56–>100	3.13	25
Escherichia coli (107)	KP-736	≤0.006–1.56	0.013	0.10
	Ceftazidime	≤0.006–0.78	0.10	0.39
	Cefotaxime	0.013–3.13	0.05	0.20
	Cefpirome	≤0.006–0.39	0.025	0.05
Citrobacter freundii (96)	KP-736	≤0.006–25	0.025	3.13
	Ceftazidime	0.05–>100	0.39	100
	Cefotaxime	0.05–>100	0.20	50
	Cefpirome	0.025–100	0.05	3.13
Klebsiella pneumoniae (127)	KP-736	≤0.006–0.39	0.013	0.025
	Ceftazidime	0.025–6.25	0.10	0.20
	Cefotaxime	0.013–1.56	0.05	0.10
	Cefpirome	0.013–3.13	0.05	0.10
Klebsiella oxytoca (100)	KP-736	≤0.006–0.10	0.013	0.025
	Ceftazidime	0.013–0.78	0.05	0.10
	Cefotaxime	≤0.006–0.39	0.025	0.05
	Cefpirome	≤0.006–1.56	0.025	0.05
Enterobacter aerogenes (10)	KP-736	0.05–6.25	0.05	0.10
	Ceftazidime	0.10–12.5	0.10	0.20
	Cefotaxime	0.05–6.25	0.10	0.10
	Cefpirome	0.025–0.39	0.05	0.05
Enterobacter cloacae (103)	KP-736	≤0.006–25	0.39	6.25
	Ceftazidime	0.05–>100	0.39	50
	Cefotaxime	0.025–>100	0.39	100
	Cefpirome	0.013–6.25	0.10	3.13
Serratia marcescens (121)	KP-736	≤0.006–>100	0.05	100
	Ceftazidime	0.10–>100	0.78	>100
	Cefotaxime	0.10–>100	1.56	>100
	Cefpirome	0.025–>100	0.20	50
Proteus mirabilis (102)	KP-736	≤0.006–1.56	0.013	0.05
	Ceftazidime	0.025–0.78	0.05	0.10
	Cefotaxime	≤0.006–0.10	0.025	0.05
	Cefpirome	≤0.025–0.39	0.05	0.10

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TABLE 1—*Continued*

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Proteus vulgaris (57)	KP-736	≤0.006–0.78	0.025	0.20
	Ceftazidime	0.05–3.13	0.10	0.20
	Cefotaxime	0.025–6.25	0.20	1.56
	Cefpirome	0.025–6.25	0.20	1.56
Morganella morganii (36)	KP-736	0.013–1.56	0.05	0.39
	Ceftazidime	0.10–12.5	0.39	3.13
	Cefotaxime	0.025–12.5	0.20	3.13
	Cefpirome	0.013–0.20	0.025	0.10
Providencia rettgeri (54)	KP-736	≤0.006–12.5	0.013	0.39
	Ceftazidime	≤0.006–3.13	0.10	0.39
	Cefotaxime	≤0.006–1.56	0.013	0.20
	Cefpirome	≤0.006–0.78	0.025	0.20
Providencia stuartii (57)	KP-736	≤0.006–25	0.013	0.20
	Ceftazidime	0.05–0.78	0.10	0.39
	Cefotaxime	≤0.006–0.78	0.05	0.20
	Cefpirome	0.013–0.39	0.05	0.20
Salmonella spp. (100)	KP-736	≤0.006–0.025	≤0.006	0.025
	Ceftazidime	0.10–0.78	0.20	0.39
	Cefotaxime	0.05–0.39	0.10	0.20
	Cefpirome	0.025–0.20	0.05	0.10
Shigella spp. (102)	KP-736	≤0.006–3.13	0.025	1.56
	Ceftazidime	0.05–0.39	0.05	0.20
	Cefotaxime	≤0.006–0.10	0.025	0.05
	Cefpirome	≤0.006–0.10	0.025	0.10
Haemophilus influenzae (95)	KP-736	0.013–12.5	0.10	0.39
	Ceftazidime	0.013–12.5	0.10	0.39
	Cefotaxime	≤0.006–3.13	0.025	0.05
	Cefpirome	≤0.006–12.5	0.05	0.39
Neisseria gonorrhoeae (23)	KP-736	0.05–1.56	0.78	1.56
	Ceftazidime	0.013–0.78	0.10	0.39
	Cefotaxime	≤0.006–0.20	0.05	0.10
	Cefpirome	≤0.006–0.39	0.05	0.20
Branhamella catarrhalis (38)	KP-736	0.39–12.5	1.56	6.25
	Ceftazidime	0.013–0.10	0.05	0.10
	Cefotaxime	0.025–0.78	0.10	0.39
	Cefpirome	0.025–1.56	0.20	0.78
Pseudomonas aeruginosa (281)	KP-736	0.013–>100	0.39	12.5
	Ceftazidime	0.39–>100	3.13	50
	Cefotaxime	3.13–>100	50	>100
	Cefpirome	0.78–>100	12.5	100
Pseudomonas aeruginosa, imipenem resistant (73)	KP-736	0.05–>100	1.56	12.5
	Ceftazidime	0.78–>100	25	50
	Cefotaxime	6.25–>100	>100	>100
	Imipenem	6.25–25	12.5	25
Pseudomonas cepacia (49)	KP-736	≤0.006–3.13	0.025	0.39
	Ceftazidime	0.78–>12.5	1.56	3.13
	Cefotaxime	3.13–>100	6.25	25
	Cefpirome	3.13–>100	12.5	25
Xanthomonas maltophilia (96)	KP-736	0.05–>100	1.56	25
	Ceftazidime	1.56–>100	50	>100
	Cefotaxime	3.13–>100	100	>100
	Cefpirome	6.25–>100	100	>100
Acinetobacter calcoaceticus (83)	KP-736	0.025–100	0.78	12.5
	Ceftazidime	0.78–25	6.25	25
	Cefotaxime	1.56–>100	25	50
	Cefpirome	0.39–100	3.13	12.5

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TABLE 1—*Continued*

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Clostridium difficile (21)	KP-736	25–50	25	25
	Ceftazidime	25–100	100	100
	Cefotaxime	25–50	50	50
	Cefpirome	25–25	25	25
Clostridium perfringens (16)	KP-736	0.10–1.56	0.39	0.78
	Ceftazidime	0.025–0.20	0.05	0.10
	Cefotaxime	0.013–0.20	0.025	0.05
	Cefpirome	≤0.006–0.10	0.025	0.10
Bacteroides fragilis (51)	KP-736	6.25–>100	50	>100
	Ceftazidime	3.13–>100	25	>100
	Cefotaxime	0.39–>100	6.25	100
	Cefpirome	0.39–>100	25	>100

Time-kill study. The mid-logarithmic-phase cells (approximately 10^6 CFU/ml) were exposed to antibiotics at a concentration of 1/4, 1/2, 1, 2, or 4 MICs. Two 50-μl samples were removed at fixed times, and several 10-fold dilutions were prepared in saline if needed and plated onto antibiotic-free SDA-N (10 ml per plate). Drug carryover was reduced by a 200-fold dilution of the sample with agar and did not affect colony formation. The number of colonies was counted after a 24-h incubation at 37°C. We could detect viable cells above 20 CFU/ml by this procedure.

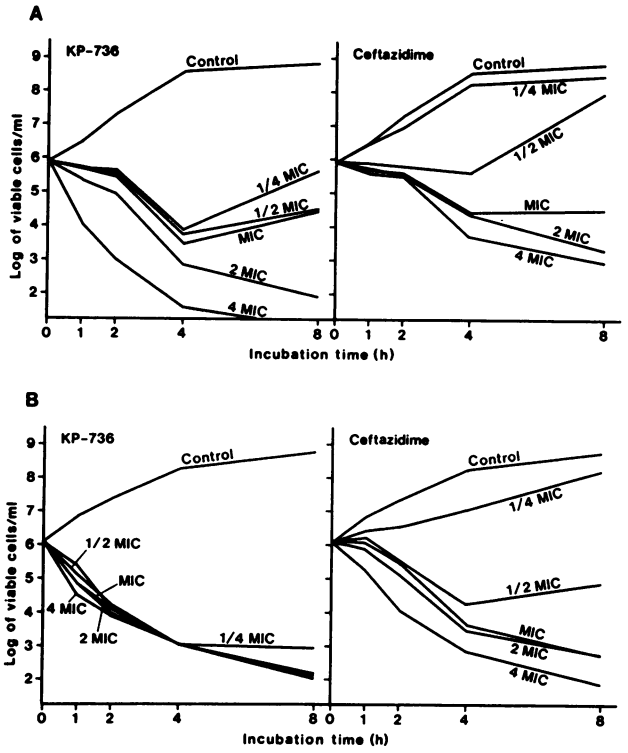

FIG. 2. Bactericidal activities of KP-736 and ceftazidime against *E. coli* K-12 strain C600 (A) and *P. aeruginosa* NCTC 10490 (B). The MICs of KP-736 and ceftazidime were 0.39 and 0.20 μg/ml, respectively, against *E. coli* K-12 strain C600 and 0.39 and 1.56 μg/ml, respectively, against *P. aeruginosa* NCTC 10490 by the broth dilution method.

Assay of affinity for PBPs. The affinity for PBPs was determined by the conventional competition assay with [^{14}C]penicillin G (Amersham Japan Co., Ltd., Tokyo, Japan) as described previously (20) with some modifications (21). Membrane proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by fluorography. The relative band densities on the fluorogram were determined by using a scanning densitometer (Densitron PAN-802; Jookoo, Tokyo, Japan). The binding affinities of antibiotics for each PBP were expressed in terms of the concentration required to prevent [^{14}C]penicillin G binding by 50%.

Stability to β-lactamase. The various types of β-lactamase used in this study were totally or partially purified enzymes. The stability of the antibiotics to β-lactamase was determined by the spectrophotometric assay (22). The molecular absorbancy difference ($\Delta\varepsilon$) and the specific wavelength for KP-736 were 6.65/mM/cm and 272.7 nm, respectively. The relative rate of hydrolysis was determined as the initial rate at 100 μM each compound.

RESULTS

Antibacterial activity. The in vitro activities of KP-736, ceftazidime, cefotaxime, and cefpirome against gram-positive and gram-negative bacteria are summarized in Table 1. KP-736 has a broad antibacterial spectrum and was highly active against gram-negative bacteria, especially against glucose nonfermenters, including the *Pseudomonas* group. For gram-positive bacteria, cefotaxime and cefpirome were highly active against *Streptococcus pneumoniae* and *Streptococcus pyogenes* and KP-736 was less active against staphylococci and *Enterococcus faecalis*. KP-736 was less active against the anaerobes tested, but its activity was comparable to that of cefpirome against *Clostridium difficile* and *Bacteroides fragilis*. KP-736 was significantly more active in terms of the MIC for 50% of isolates (MIC_{50}) against all members of the family *Enterobacteriaceae* tested, except *Enterobacter* and *Shigella* spp., than were the reference drugs, although its MIC for 90% of isolates (MIC_{90}) was substantially higher than those of the reference drugs. KP-736 had activity similar to that of cefpirome or was 1 or 2 dilutions more active at lower concentrations against members of the *Enterobacteriaceae* such as *Escherichia coli*, *Klebsiella oxytoca*, *Serratia marcescens*, *Proteus* spp., and *Providencia* spp., but excluding *Enterobacter* spp. and *Mor-*

TABLE 2. Affinities of KP-736 for PBPs of *P. aeruginosa* NCTC 10490 and *E. coli* K-12 strain C600

Organism	Antibiotic	IC_{50} (μg/ml) with PBP:						MIC[a] (μg/ml)
		1A	1B(s)	2	3	4	5(/6)	
P. aeruginosa NCTC 10490	KP-736	0.058	1.48	40.4	0.081	0.82	>100	0.39
	Ceftazidime	0.12	10.2	>100	0.081	1.83	>100	1.56
	Cefotaxime	0.041	0.92	>100	0.049	1.30	>100	25
	Cefpirome	0.068	6.58	>100	0.26	0.23	>100	12.5
E. coli K-12 strain C600	KP-736	0.33	0.96	1.11	0.073	24.0	>100	0.39
	Ceftazidime	1.22	2.71	67.7	0.096	>100	>100	0.20
	Cefotaxime	0.11	0.39	3.93	0.14	14.3	>100	0.10
	Cefpirome	6.50	2.64	9.72	0.097	15.9	>100	0.10

[a] The MICs were determined by the broth dilution method with a final inoculum of about 10^6 CFU/ml.

ganella morganii. The MIC_{50}s of KP-736 against *Neisseria gonorrhoeae* and *Branhamella catarrhalis* were 0.78 and 1.56 μg/ml, respectively, although reference compounds inhibited 90% of the organisms at the concentrations.

The most characteristic activity of KP-736 is its superior activity against *Pseudomonas* spp. Its MIC_{50} for *P. aeruginosa* was 0.39 μg/ml, its activity was four- to eightfold higher than that of ceftazidime, and its MIC_{90} was equal to the MIC_{50} of cefpirome, 12.5 μg/ml. The MIC_{90} of KP-736 for imipenem-resistant *P. aeruginosa* was identical to that for 281 susceptible strains, although the MIC_{50} for the resistant strains was fourfold higher than that for the susceptible strains. Furthermore, *Pseudomonas cepacia* was surprisingly susceptible to KP-736, which had an MIC_{90} of 0.39 μg/ml, although 0.39 μg of control drugs per ml failed to inhibit 50% of the strains. KP-736 was also highly active against *Xanthomonas maltophilia*, with an MIC_{50} of 1.56 μg/ml, while reference compounds had lower activity against the strain. Cefotaxime was the least active compound tested against the *Pseudomonas* group.

Time-kill study. The bactericidal activities of KP-736 were compared with those of ceftazidime against *E. coli* K-12 strain C600 and *P. aeruginosa* NCTC 10490 (Fig. 2). In broth dilution experiments, the MICs of KP-736 and ceftazidime were 0.39 and 0.20 μg/ml against *E. coli* at 9.2×10^5 CFU/ml and 0.39 and 1.56 μg/ml against *P. aeruginosa* at 5.0×10^5 CFU/ml, respectively. Time-kill studies were carried out at about 10^6 CFU/ml, which was a slightly larger inoculum than that for the MIC determination. KP-736 was bactericidal against *E. coli* even at 1/4 MIC for 4 h, whereas ceftazidime failed to reduce the number of CFU at 1/4 MIC. In addition, there was an evident difference in bactericidal activity between KP-736 and ceftazidime at concentrations higher than the MICs. There was no significant difference in the reduction in the number of cells of these two strains by ceftazidime. On the other hand, KP-736 expressed equally potent bactericidal activity against *P. aeruginosa* at every dose tested; 1/4 MIC of KP-736 was enough to reduce the CFU by 3 logs in 4 h.

Affinity for PBPs. The affinities of KP-736 for PBPs of *E. coli* K-12 strain C600 and *P. aeruginosa* NCTC 10490 were estimated by the conventional competition assay with [^{14}C]penicillin G (Table 2). KP-736 had very high affinities for PBP 3 of *E. coli* and PBP 1A and PBP 3 of *P. aeruginosa*, as did cefotaxime. The affinities of the test compounds for PBP 5/6 of *E. coli* and PBP 5 of *P. aeruginosa* were poor. The concentration required to prevent [^{14}C]penicillin binding by 50% (IC_{50}) was lower for KP-736 than for the reference

TABLE 3. Stability of KP-736 to various β-lactamases

Enzyme source	Type of β-lactamase[a]	Relative rate of hydrolysis[b] of:			
		KP-736	Ceftazidime	Cefotaxime	Cefpirome
E. coli GN5482	CSase (Ia)	0.12	<0.10	0.14	0.35
C. freundii GN7391	CSase (Ia)	0.28	<0.10	<0.10	0.32
E. cloacae GN7471	CSase (Ia)	0.18	<0.10	<0.10	0.19
S. marcescens GN10857	CSase (Ia)	0.14	0.39	0.63	3.7
M. morganii GN5407	CSase (Ia)	0.41	0.58	0.36	0.79
P. rettgeri GN4430	CSase (Ia)	6.1	0.53	1.3	0.55
P. aeruginosa GN10362	CSase (Ia)	0.21	0.10	2.5	0.79
K. oxytoca GN10650	CXase type I (IV)	4.1	0.17	25	4.8
P. vulgaris GN7919	CXase type I (Ic)	25	1.4	19	6.4
P. cepacia GN11164	CXase type I (Ic)	26	1.5	48	29
X. maltophilia GN12873(L-2)	CXase type I	9.6	2.7	7.9	23
X. maltophilia GN12873(L-1)	CXase type II	21	19	184	49
E. coli ML4901(Rms212)	PCase type I (IIIa)	0.21	<0.10	0.11	0.17
E. coli ML4901(Rms213)	PCase type II (Va)	5.6	<0.10	9.6	55
E. coli ML4901(Rte161)	PCase type III (Vb)	0.40	<0.10	0.16	0.11
E. coli ML4901(Rms149)	PCase type IV (Vc)	0.25	<0.10	<0.10	<0.10
S. aureus MS15009(pI258)	PCase type V	0.17	<0.10	0.14	<0.10

[a] Abbreviations: CSase, cephalosporinase; PCase, penicillinase. The Richmond and Sykes classification (17a) is also shown in parentheses.

[b] Relative rate of hydrolysis is expressed as the percentage of hydrolysis of the substrate (100 μM). As a substrate, penicillin G was used for penicillinases and cephaloridine was used for the others.

drugs for PBP 2 of both organisms, although the IC_{50} was higher than the MIC.

Stability to β-lactamase. The stability of KP-736 to enzymatic hydrolysis was tested with various types of β-lactamases. Hydrolysis of test compounds was expressed as the rate of hydrolysis relative to cephaloridine or penicillin G as 100 (Table 3). KP-736 was stable to all the cephalosporinases tested, as was cefpirome, except for *Providencia rettgeri*. KP-736 was slightly hydrolyzed by the oxyiminocephalosporinases (CXases), especially by the enzymes from *Proteus vulgaris* and *Pseudomonas cepacia*. Reference compounds were also hydrolyzed by CXases to some extent. Ceftazidime was more stable than KP-736 to all the enzymes tested, with the exception of cephalosporinases from *Serratia marcescens* and *M. morganii*, to which their stabilities were equivalent. KP-736, cefotaxime, and cefpirome were slightly hydrolyzed by type II penicillinase, to which the latter was the most unstable. KP-736 was stable to the other types of penicillinases, including type V penicillinase from *Staphylococcus aureus*.

DISCUSSION

In the past decade, cephalosporins have been endowed with excellent antibacterial activities as a consequence of modifications of the compounds (15). However, the clinical usefulness of these antibiotics has been limited owing to the rapid development of resistance of bacteria, such as *Staphylococcus aureus*, *Enterobacter cloacae*, *Citrobacter freundii*, *Serratia marcescens*, and *P. aeruginosa* (17–19). The aminothiazolyl side chain at C-7 of the cephem nucleus is generally seen in the structure of the broad-spectrum cephalosporins. KP-736 differs from those drugs in that it has the novel substituent, 1,5-dihydroxy-4-pyridone group, at the C-7 position. In the present study we found that KP-736 was superior to the reference compounds tested in its antibacterial activities against most members of the family *Enterobacteriaceae* and glucose nonfermenters including *P. aeruginosa*. Furthermore, KP-736 was effective against the strains resistant to cefotaxime, ceftazidime, or imipenem. KP-736 is the aminothiazolyl oxyiminocephalosporin with the thiadiazole-thiomethyl side chain at the 3-position, as in cefzonam. Hikida et al. have suggested that the presence of 1,2,3-thiadiazolyl group at the 3-position side chain confers good activity against gram-positive bacteria on aminothiazolyl cephalosporin (4). This thiadiazolyl substituent seems to fail to provide KP-736 with activity similar to that of cefzonam against gram-positive bacteria, especially *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Enterococcus faecalis*. However, the poor activities against methicillin-resistant *Staphylococcus aureus* and *Enterococcus faecalis* were deficiencies that KP-736 shares with the reference compounds.

In this study we also found that KP-736 had potent bactericidal activities against *E. coli* and *P. aeruginosa*, even when it was present at concentrations below the MIC. Furthermore, KP-736 reduced the viable cell count of *P. aeruginosa* for 8 h at any concentration tested, by 3 logs at 1/4 MIC and 4 logs at 1/2 MIC or above the MIC. The binding affinities of KP-736 for PBP 1A and PBP 1B(s) of these strains were higher than those of ceftazidime, although those for PBP 3 were comparable. It appears that 1/4 MIC is enough for KP-736 to saturate the target, PBPs, and express bactericidal action for 8 h. Taking into consideration that cefotaxime was less active than KP-736 against these strains despite having similar binding activities for PBPs, we suppose that KP-736 passes through the outer membrane of bacterial cells more easily than cefotaxime does.

Recently, new β-lactams that possess two adjacent hydroxy group or surrogate groups in common on the side chain were synthesized that had good activity against gram-negative bacteria including *P. aeruginosa* in spite of different structures of the β-lactam nucleus and positions of the side chains (1, 3, 6, 10, 11, 13, 14, 16). Some of these compounds, such as E-0702 (23), M14659 (12), and pirazmonam (2), have been reported to chelate iron and have been suggested to be incorporated into bacterial cells via the *tonB*-dependent iron transport system. It has been demonstrated that KP-736 similarly chelates iron and exhibits strong activities against laboratory mutants of *P. aeruginosa* with an altered outer membrane, and it was suggested that the compound was incorporated into the cells of *P. aeruginosa* not only through the porin but also by the iron transport proteins (25). This feature enables us to understand the excellent activity of KP-736 mentioned above.

Mitsuhashi et al. have proposed the classification of a group of enzymes, CXases, in terms of substrate specificity and have divided them into two subgroups, types I and II, by substrate and inhibitor profiles (9). The potent activities of KP-736 were also effective against bacteria that produce CXases, such as *K. oxytoca*, *Proteus vulgaris*, *Pseudomonas cepacia*, and *Xanthomonas maltophilia*, although KP-736 was hydrolyzed by the enzymes from these species to some extent. This also suggests that hydrolysis of KP-736 by the enzymes is overcome by rapid supply of the drug resulting from the special penetration or by high binding affinity to the target, PBPs. KP-736 is stable to hydrolysis by penicillinases and cephalosporinases; this stability is comparable to that of cefotaxime. This characteristic may also raise the activity of KP-736 against gram-negative clinical isolates that produce chromosome- or plasmid-mediated β-lactamase.

Since KP-736 has strong activity against clinical isolates, including strains resistant to imipenem, we consider that KP-736 is a very promising parenteral cephalosporin for the treatment of serious infection caused by gram-negative bacteria.

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