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In Vitro and In Vivo Antibacterial Activities of ER-35786, a New Antipseudomonal Carbapenem

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ER-35786 is a new parenteral 1β-methyl carbapenem with a broad antibacterial spectrum and a potent antipseudomonal activity. It showed high in vitro activity, comparable to those of meropenem and a new carbapenem, BO-2727, against methicillin-susceptible *Staphylococcus aureus* and streptococci, with MICs at which 90% of strains tested are inhibited (MIC_{90}s) of ≤0.39 μg/ml. Against methicillin-resistant *S. aureus*, ER-35786 was the most active among the compounds tested, yet its MIC_{90} was 12.5 μg/ml. Against members of the family *Enterobacteriaceae*, *Moraxella catarrhalis*, and *Haemophilus influenzae*, ER-35786 inhibited 90% of strains tested at a concentration of ≤1.56 μg/ml. The MIC_{90} of ER-35786 for *Pseudomonas aeruginosa* was 3.13 μg/ml, and the compound was more active than meropenem. In addition, the activity of ER-35786 against imipenem-, meropenem-, cefclidin-, or ceftazidime-resistant *P. aeruginosa* was equal to or higher than that of the most active reference compound. The in vivo activity of ER-35786 was consistent with this in vitro activity. The in vivo activity of ER-35786 was highest for systemic infection models with methicillin-resistant *S. aureus* and β-lactam-resistant *P. aeruginosa* strains. In acute pneumonia caused by *P. aeruginosa*, ER-35786 produced a greater reduction in the viable cell count in the lungs than did imipenem-cilastatin or meropenem.

Pseudomonas aeruginosa is one of the most important causes of infection in immunosuppressed patients and is also a pathogen that causes chronic infectious diseases. It is resistant to many antimicrobial compounds because of the low permeability of its outer membrane. Some recently developed antibiotics, such as carbapenems and "fourth-generation" cephalosporins which have a broad spectrum of potent antibacterial activities and low affinity for and high-level resistance to β-lactamases, such as cefpirome, possess antipseudomonal activity, but resistant strains continue to emerge. In the course of our research directed toward the development of new antibiotics which show improved antipseudomonal activity, ER-35786 (Fig. 1) was synthesized. ER-35786, (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-{(2*S*,4*S*)-[(3*R*)-pyrrolidin-3-yl-(*R*)-hydroxymethyl]pyrrolidin-4-ylthio}-1-carbapen-2-em-3-carboxylic acid hydrochloride, is a new injectable carbapenem which has a methyl group at the 1β position. In the study described in this report, we compared the in vitro and in vivo activities of ER-35786 with those of imipenem (13), meropenem (19), a new carbapenem, BO-2727 (12), cefclidin (24), cefluprenam (25), and ceftazidime (27).

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MATERIALS AND METHODS

Antibiotics. ER-35786, cefclidin (24), cefluprenam (25), meropenem, and BO-2727 (12) were synthesized at Tsukuba Research Laboratories, Eisai Co., Ltd. The following antibiotics were obtained commercially: imipenem-cilastatin (Tienam; Banyu Pharmaceutical Co., Ltd., Tokyo, Japan), penicillin G (Banyu Pharmaceutical Co., Ltd., Tokyo, Japan), ceftazidime (Modacin; Nippon Glaxo Co., Ltd., Tokyo, Japan), ofloxacin (Tarivid; Daiichi Pharmaceuticals, Tokyo, Japan), cephalothin (Keflin; Shionogi & Co., Ltd., Osaka, Japan), cephaloridine (Keflodin; Shionogi & Co., Ltd., Osaka, Japan), and cefsulodin (Takesulin; Takeda Chemical Industries, Ltd., Osaka, Japan). Ofloxacin was extracted from Tarivid, and imipenem and cilastatin were separated from Tienam at Tsukuba Research Laboratories, Eisai Co., Ltd.

Organisms. The bacterial strains used for susceptibility testing were clinical isolates obtained from various hospitals in Japan between 1989 and 1994 and were unselected. Other strains were chosen for testing of β-lactamase production. Included in this group were 10 laboratory strains, kindly provided by S. Mitsuhashi (Episome Institute, Gunma, Japan) and T. Sawai (Chiba University, Chiba, Japan). Isogenic *P. aeruginosa* strains resistant to β-lactams were also used. Strain E03441 R24, deficient in OprD protein, and strain E03441 SKR2, derepressed for group 1 β-lactamase production, were described previously (26). Spontaneous meropenem-resistant mutants were selected from strains E03441 WT (parent strain) on agar medium that contained 6.25 μg of meropenem per ml, and one representative meropenem-resistant mutant was designated E03441 MR-3. Strain E03441 OCR-5, an OprM mutant, was isolated from strain E03441 WT by selection for ofloxacin and cefsulodin resistance (9). OprD-deficient and group 1 β-lactamase-overproducing mutant strain E03441 SKR2/I was isolated from strain E03441 SKR2 by selection for imipenem resistance. Group 1 β-lactamase-nonproducing mutant strain E03441 SKR2-14 was isolated from strain E03441 SKR2 by treatment with ethyl methanesulfonate (7). Group 1 β-lactamase-nonproducing and OprD-deficient mutant E03441 SKR2/I-2 was isolated from strain E03441 SKR2/I by treatment with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (7). All isolates were maintained as stock cultures in our laboratory.

Determination of MICs. The MICs of the compounds were determined by an agar dilution method with an inoculum of 10^4 CFU/spot. Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) was used for all organisms except the following: streptococci (Mueller-Hinton agar supplemented with 5% defibrinated sheep blood), *Haemophilus influenzae* and *Moraxella catarrhalis* (haemophilus test agar medium [3]), and obligate anaerobes (GAM agar medium; Nissui Pharmaceutical Co., Tokyo, Japan). Agar plates were incubated for 18 to 24 h at 30°C for *Burkholderia cepacia* and *Stenotrophomonas maltophilia* and at 37°C for the other bacteria tested. For anaerobes, incubation was carried out anaerobically in GasPak jars (BBL), and for streptococci, *H. influenzae*, and *M. catarrhalis*, incubation was carried out in an atmosphere of 5% CO_2. The MIC was taken to be the lowest concentration that completely inhibited visible growth on agar plates. Selected strains were also tested by a microdilution method with cation-supplemented Mueller-Hinton broth (BBL). Final inocula of 5×10^5 CFU/ml were prepared by diluting overnight cultures into fresh broth medium. The MIC was determined by visual inspection for a lack of turbidity after 24 h of incubation at 37°C. Samples (2 μl) from clear wells were transferred to antibiotic-free plates. The minimum bactericidal concentration (MBC), defined as a 99.9% reduction in the numbers of CFU relative to the numbers in the inoculum, was determined by the method of Pearson et al. (16).

β-Lactamase activity. Various β-lactamases were prepared as described previously (4). β-Lactamase hydrolysis studies were performed spectrophotometrically on a Hitachi U-3000 spectrophotometer, as described previously (25). The substrate concentration used was 100 μM. The enzyme reaction was carried out

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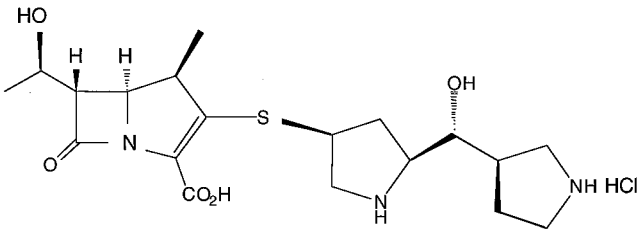

FIG. 1. Chemical structure of ER-35786, (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-{(2*S*,4*S*)-[(3*R*)-pyrrolidin-3-yl-(*R*)-hydroxymethyl]pyrrolidin-4-ylthio}-1-carbapen-2-em-3-carboxylic acid hydrochloride.

at 30°C. The detection wavelengths and molar extinction coefficients ($\Delta\varepsilon$) for the compounds used in this study were as follows: for ER-35786, 297 nm and $\Delta\varepsilon$ = 7.25 mM^{-1} cm^{-1}; for imipenem, 299 nm and $\Delta\varepsilon$ = 8.00 mM^{-1} cm^{-1} (17); for meropenem, 299 nm and $\Delta\varepsilon$ = 7.37 mM^{-1} cm^{-1}; for cephaloridine, 260 nm and $\Delta\varepsilon$ = 10.2 mM^{-1} cm^{-1} (10); for cephalothin, 262 nm and $\Delta\varepsilon$ = 7.66 mM^{-1} cm^{-1} (10); and for penicillin G, 233 nm and $\Delta\varepsilon$ = 1.14 mM^{-1} cm^{-1} (10).

Systemic infections in mice. Systemic infections were induced as described previously (20). The test organisms were cultured overnight at 37°C on brain heart infusion agar (BHIA; Difco Laboratories, Detroit, Mich.). Four-week-old male ICR mice (weight, 25 to 28 g; 10 mice in each group; Charles River Japan Inc., Kanagawa, Japan) were injected intraperitoneally with bacterial suspensions containing 5% mucin (Difco). The test compounds, ER-35786, imipenem-cilastatin, meropenem, BO-2727, and cefclidin, were administered subcutaneously once at 1 h after infection. For *Staphylococcus aureus*, cefluprenam was used instead of cefclidin. The survival of the infected mice was monitored for 1 week, and the 50% effective dose (ED_{50}) was determined from the final survival rate by the Litchfield-Wilcoxon method (6).

Acute pneumonia model in mice. Acute pneumonia in mice was induced as described previously (21). Four-week-old male ICR mice were injected intraperitoneally with 200 mg of 5-fluorouracil (5-FU; Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan) per kg of body weight. By this treatment, the number of neutrophils in the peripheral blood is reduced to less than 10% of normal levels from 6 to 12 days after 5-FU administration (21). After 6 days, mice (weight, 26 to 28 g; five mice in each group) were anesthetized intravenously with ketamine and were challenged intranasally with *P. aeruginosa* organisms suspended in saline. The test organisms were cultured overnight at 37°C on BHIA. The test compounds were administered subcutaneously eight times at 1-h intervals after infection. At 1 h after the final administration, the lungs were removed aseptically under diethyl ether anesthesia and were homogenized in saline. The homogenate was mixed with modified Drigalski agar (Eiken Chemicals Co., Tokyo, Japan). The CFU was enumerated and was regarded as the number of viable cells after overnight incubation at 37°C. The lower limit of detection was 3 CFU/lung. The observed differences in efficacy were evaluated statistically by using JMP software (SAS Institute Inc., Cary, N.C.).

Susceptibility to renal dehydropeptidase I (DHP-I) hydrolysis. The fresh kidneys of animals and humans were minced and homogenized with 4 volumes of 50 mM MES [2-(*N*-morpholino)ethanesulfonic acid] buffer (pH 7.4) and were stored at −80°C until use. Each compound was dissolved in 50 mM MES buffer at a concentration of 1 mg/ml, and 0.6 ml of the solutions were mixed with 0.6 ml of the kidney homogenates described above. At intervals during incubation for 120 min at 37°C, samples (0.1 ml) were withdrawn, added to 1.0 ml of acetonitrile at 0°C to remove the proteins, and centrifuged. The extracts that were obtained were diluted with 4 ml of 50 mM MES buffer, 50 μl of the diluents was subjected to high-performance liquid chromatography, and the residual amounts of the compounds were determined. The susceptibility of each compound is expressed as the relative half-life ($t_{1/2}$) of hydrolysis, with the absolute $t_{1/2}$ of meropenem taken as 1.

RESULTS

Antibacterial activity. The in vitro antibacterial activity of ER-35786 against 21 species of clinical isolates was compared with those of imipenem, meropenem, BO-2727, cefclidin, and ceftazidime (Table 1). In tests with gram-positive bacteria, cefluprenam was also examined. The MIC of ER-35786 at which 90% of isolates are inhibited (MIC_{90}) for methicillin-susceptible *S. aureus* was 0.10 μg/ml, and it was four times higher than the MIC_{90} of imipenem and half that of meropenem. Against methicillin-resistant *S. aureus*, ER-35786 was four to eight times more active than imipenem and meropenem, but this activity (MIC_{90}, 12.5 μg/ml) was weaker than that against methicillin-susceptible *S. aureus*. ER-35786 was highly active against *Streptococcus pyogenes*, as were the reference carbapenems (MIC_{90}, ≤0.006 μg/ml). ER-35786 was also active against *Streptococcus pneumoniae* (MIC_{90}, 0.39 μg/ml), yet it was two to four times less active than imipenem and meropenem. Of 10 strains of *S. pneumoniae* tested, two were resistant to penicillin G (MICs, 1.56 μg/ml) and two were moderately resistant to penicillin G (MICs, 0.10 to 0.39 μg/ml). Against *Enterococcus faecalis*, the activity of ER-35786 (MIC_{90}, 12.5 μg/ml) was comparable to those of imipenem and meropenem. In addition, carbapenems including ER-35786 were more active against gram-positive cocci than the cephalosporins tested.

ER-35786 was highly active against most members of the family *Enterobacteriaceae*, 90% of which were inhibited by ER-35786 at a concentration of 1.56 μg/ml. The MIC_{90}s of ER-35786 for *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Citrobacter freundii* were 0.05 to 0.20 μg/ml; its activity was two to four times higher than that of imipenem and comparable to or two times less than that of meropenem. In particular, ER-35786 was at least 16 times more active against *E. cloacae* and *C. freundii* than the cephalosporins cefclidin and ceftazidime, and no cross-resistance between ER-35786 and cephalosporins was observed. While ER-35786 was also active against *Serratia marcescens*, the susceptibilities of 3 of 56 strains tested not only to ER-35786 (MICs, 6.25 to 25 μg/ml) but also to all the reference compounds were greatly reduced. For *Proteus mirabilis*, *Proteus vulgaris*, *Providencia rettgeri*, and *Morganella morganii*, the MIC_{90}s of ER-35786 were 0.78, 1.56, 1.56, and 1.56 μg/ml, respectively, and this activity was similar to those of imipenem and BO-2727 but 4 to 16 times lower than those of meropenem and cefclidin.

ER-35786 showed high activity against *M. catarrhalis* MIC_{90}, 0.05 μg/ml, and its activity was four times higher than that of imipenem but was four times lower than that of meropenem. The activity of ER-35786 against *H. influenzae* was lower than that against *M. catarrhalis*, but its MIC_{90} was 1.56 μg/ml. Of 39 strains of *H. influenzae* tested, 7 were resistant to imipenem (MICs, >12.5 μg/ml; 3 of the 7 strains were also less susceptible to ER-35786 (MICs, 1.56 to 3.13 μg/ml), but four remained susceptible to ER-35786, with MICs of 0.39 μg/ml.

An important feature of ER-35786 is its activity against *P. aeruginosa*. The MIC_{90} of ER-35786 for *P. aeruginosa* was 3.13 μg/ml, and its activity was two to four times higher than those of imipenem, meropenem, BO-2727, and cefclidin and 16 times higher than that of ceftazidime. Against β-lactam-resistant *P. aeruginosa*, ER-35786 showed activity comparable to or higher than those of the most active reference compounds. However, the activity of ER-35786 against β-lactam-resistant *P. aeruginosa* was lower than that against β-lactam-susceptible *P. aeruginosa*, as were those of the reference carbapenems. Against imipenem-resistant *P. aeruginosa* (MIC, ≥12.5 μg/ml), ER-35786, with an MIC_{90} of 6.25 μg/ml, was about four times more active than imipenem and meropenem and was twice as active as BO-2727. Against meropenem-resistant *P. aeruginosa*, ER-35786, with an MIC_{90} of 6.25 μg/ml, was four times more active than imipenem and meropenem. Against cefclidin- or ceftazidime-resistant *P. aeruginosa*, ER-35786 was 4 times more active than imipenem, meropenem, and cefclidin and 16 times more active than ceftazidime.

ER-35786 showed weak activity against *B. cepacia* (MIC_{90}, 6.25 μg/ml), as did the reference carbapenems. ER-35786 was as inactive (MIC_{90}, >100 μg/ml) against *S. maltophilia* as the reference carbapenems. However, against *Acinetobacter calcoaceticus*, ER-35786 was as active, with an MIC_{90} of 0.39

TABLE 1. Antibacterial activities of ER-35786 and other compounds against clinical isolates

Organism (no. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus aureus, methicillin susceptible (29)	ER-35786	0.05–0.20	0.05	0.10
	Imipenem	0.012–0.05	0.025	0.025
	Meropenem	0.10–0.39	0.10	0.20
	BO-2727	0.05–0.20	0.05	0.10
	Cefclidin	6.25–25	6.25	25
	Ceftazidime	6.25–25	6.25	12.5
	Cefluprenam	0.39–0.78	0.78	0.78
Staphylococcus aureus, methicillin resistant (24)	ER-35786	0.39–12.5	6.25	12.5
	Imipenem	0.025–100	12.5	100
	Meropenem	0.78–50	12.5	50
	BO-2727	0.39–25	6.25	25
	Cefclidin	12.5–>100	100	>100
	Ceftazidime	50–>100	>100	>100
	Cefluprenam	1.56–100	50	100
Streptococcus pyogenes (11)	ER-35786	≤0.006	≤0.006	≤0.006
	Imipenem	≤0.006	≤0.006	≤0.006
	Meropenem	≤0.006	≤0.006	≤0.006
	BO-2727	≤0.006	≤0.006	≤0.006
	Cefclidin	0.05–0.10	0.05	0.10
	Ceftazidime	0.10–0.20	0.10	0.20
	Cefluprenam	0.025	0.025	0.025
Streptococcus pneumoniae (10)	ER-35786	≤0.006–0.39	0.012	0.39
	Imipenem	≤0.006–0.10	≤0.006	0.10
	Meropenem	≤0.006–0.20	0.012	0.20
	BO-2727	0.012–0.39	0.012	0.39
	Cefclidin	0.20–6.25	0.39	3.13
	Ceftazidime	0.10–6.25	0.78	6.25
	Cefluprenam	0.012–0.39	0.20	0.39
Enterococcus faecalis (14)	ER-35786	1.56–12.5	3.13	12.5
	Imipenem	0.78–6.25	0.78	6.25
	Meropenem	3.13–25	6.25	25
	BO-2727	1.56–12.5	3.13	12.5
	Cefclidin	100–>100	100	>100
	Ceftazidime	>100	>100	>100
	Cefluprenam	1.56–>100	25	25
Escherichia coli (12)	ER-35786	0.012–0.05	0.025	0.05
	Imipenem	0.05–0.20	0.10	0.20
	Meropenem	0.012–0.025	0.012	0.025
	BO-2727	0.025–0.10	0.05	0.05
	Cefclidin	0.05–0.10	0.05	0.10
	Ceftazidime	0.05–0.39	0.10	0.20
Klebsiella pneumoniae (14)	ER-35786	0.05–0.20	0.05	0.10
	Imipenem	0.10–0.20	0.20	0.20
	Meropenem	0.025–0.05	0.025	0.05
	BO-2727	0.05–0.39	0.10	0.10
	Cefclidin	0.05–0.20	0.10	0.10
	Ceftazidime	0.05–0.39	0.10	0.20
Enterobacter cloacae (18)	ER-35786	0.025–0.20	0.05	0.20
	Imipenem	0.20–1.56	0.39	0.39
	Meropenem	0.025–0.78	0.05	0.39
	BO-2727	0.05–0.39	0.10	0.20
	Cefclidin	0.10–12.5	0.10	6.25
	Ceftazidime	0.20–>100	0.20	100
Citrobacter freundii (32)	ER-35786	0.025–0.39	0.05	0.20
	Imipenem	0.10–1.56	0.20	0.78
	Meropenem	0.012–0.78	0.05	0.20
	BO-2727	0.05–0.39	0.10	0.39
	Cefclidin	0.05–3.13	0.20	3.13
	Ceftazidime	0.10–>100	6.25	50

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TABLE 1—*Continued*

Organism (no. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
Serratia marcescens (56)	ER-35786	0.05–25	0.10	0.78
	Imipenem	0.10–25	0.20	0.78
	Meropenem	0.025–25	0.05	0.20
	BO-2727	0.10–25	0.20	0.78
	Cefclidin	0.05–>100	0.20	3.13
	Ceftazidime	0.05–50	0.10	3.13
Proteus mirabilis (13)	ER-35786	0.20–0.78	0.78	0.78
	Imipenem	0.78–3.13	1.56	3.13
	Meropenem	0.05–0.10	0.05	0.10
	BO-2727	0.20–1.56	0.78	1.56
	Cefclidin	0.10–0.39	0.20	0.39
	Ceftazidime	0.05–0.20	0.05	0.10
Proteus vulgaris (14)	ER-35786	0.10–1.56	0.20	1.56
	Imipenem	0.39–3.13	0.78	3.13
	Meropenem	0.025–0.20	0.05	0.10
	BO-2727	0.20–3.13	0.78	3.13
	Cefclidin	0.025–0.39	0.20	0.39
	Ceftazidime	0.05–0.10	0.05	0.10
Providencia rettgeri (12)	ER-35786	0.10–1.56	0.20	1.56
	Imipenem	0.39–1.56	0.78	1.56
	Meropenem	0.025–0.39	0.05	0.39
	BO-2727	0.20–3.13	0.39	1.56
	Cefclidin	0.025–0.39	0.025	0.10
	Ceftazidime	0.025–3.13	0.05	0.20
Morganella morganii (14)	ER-35786	0.20–1.56	0.78	1.56
	Imipenem	0.78–3.13	1.56	3.13
	Meropenem	0.05–0.20	0.10	0.10
	BO-2727	0.39–3.13	1.56	3.13
	Cefclidin	0.05–0.39	0.10	0.10
	Ceftazidime	0.05–50	0.10	12.5
Moraxella catarrhalis (15)	ER-35786	0.012–0.05	0.025	0.05
	Imipenem	0.025–0.20	0.10	0.20
	Meropenem	≤0.006–0.012	≤0.006	0.012
	BO-2727	0.025–0.10	0.05	0.10
	Cefclidin	0.78–6.25	1.56	3.13
	Ceftazidime	0.05–0.39	0.10	0.39
Haemophilus influenzae (39)	ER-35786	0.20–3.13	0.39	1.56
	Imipenem	0.39–25	1.56	12.5
	Meropenem	0.05–0.78	0.10	0.39
	BO-2727	0.20–6.25	0.78	3.13
	Cefclidin	0.10–1.56	0.20	0.78
	Ceftazidime	0.05–0.78	0.10	0.39
Pseudomonas aeruginosa (105)	ER-35786	0.10–6.25	0.39	3.13
	Imipenem	0.39–25	1.56	12.5
	Meropenem	0.025–50	0.39	6.25
	BO-2727	0.20–12.5	0.78	6.25
	Cefclidin	0.20–50	0.78	6.25
	Ceftazidime	0.39–>100	3.13	50
Pseudomonas aeruginosa, imipenem resistant (MIC, ≥12.5 μg/ml) (13)	ER-35786	1.56–6.25	3.13	6.25
	Imipenem	12.5–25	25	25
	Meropenem	3.13–50	6.25	25
	BO-2727	6.25–12.5	6.25	12.5
	Cefclidin	0.39–6.25	3.13	6.25
	Ceftazidime	0.78–100	12.5	50
Pseudomonas aeruginosa, meropenem-resistant (MIC, ≥6.25 μg/ml) (20)	ER-35786	0.20–6.25	3.13	6.25
	Imipenem	0.39–25	12.5	25
	Meropenem	6.25–50	6.25	25

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TABLE 1—*Continued*

Organism (no. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
	BO-2727	0.39–12.5	6.25	12.5
	Cefclidin	0.39–50	3.13	12.5
	Ceftazidime	1.56–>100	12.5	50
Pseudomonas aeruginosa, cefclidin-resistant (MIC, ≥6.25 μg/ml) (20)	ER-35786	0.20–6.25	0.78	6.25
	Imipenem	0.78–25	1.56	25
	Meropenem	0.20–50	1.56	25
	BO-2727	0.39–12.5	1.56	12.5
	Cefclidin	6.25–50	6.25	25
	Ceftazidime	6.25–>100	50	100
Pseudomonas aeruginosa, ceftazidime-resistant (MIC, ≥12.5 μg/ml) (29)	ER-35786	0.20–6.25	0.78	6.25
	Imipenem	0.78–25	3.13	25
	Meropenem	0.20–50	1.56	25
	BO-2727	0.39–12.5	1.56	12.5
	Cefclidin	0.78–50	6.25	25
	Ceftazidime	12.5–>100	25	100
Pseudomonas aeruginosa, ofloxacin-resistant (MIC, ≥6.25 μg/ml) (41)	ER-35786	0.20–6.25	0.39	6.25
	Imipenem	0.78–25	1.56	25
	Meropenem	0.10–50	1.56	12.5
	BO-2727	0.20–12.5	0.78	12.5
	Cefclidin	0.78–50	3.13	12.5
	Ceftazidime	0.78–>100	6.25	100
	Ofloxacin	6.25–>100	50	>100
Burkholderia cepacia (13)	ER-35786	3.13–6.25	6.25	6.25
	Imipenem	1.56–12.5	6.25	12.5
	Meropenem	0.78–3.13	1.56	3.13
	BO-2727	3.13–25	12.5	12.5
	Cefclidin	6.25–50	6.25	50
	Ceftazidime	1.56–12.5	3.13	12.5
Stenotrophomonas maltophilia (22)	ER-35786	0.78–>100	>100	>100
	Imipenem	3.13–>100	>100	>100
	Meropenem	0.39–>100	100	>100
	BO-2727	1.56–>100	>100	>100
	Cefclidin	1.56–100	12.5	50
	Ceftazidime	3.13–>100	50	>100
Acinetobacter calcoaceticus (21)	ER-35786	0.10–6.25	0.20	0.39
	Imipenem	0.10–6.25	0.20	0.39
	Meropenem	0.20–6.25	0.39	0.78
	BO-2727	0.20–6.25	0.20	0.39
	Cefclidin	0.39–25	1.56	25
	Ceftazidime	1.56–25	6.25	25
Peptostreptococcus spp. (10)	ER-35786	0.025–0.78	0.05	0.20
	Imipenem	0.025–0.39	0.05	0.10
	Meropenem	0.025–0.39	0.025	0.20
	BO-2727	0.05–0.78	0.10	0.20
	Cefclidin	0.78–12.5	3.13	6.25
	Ceftazidime	0.39–25	1.56	6.25
Bacteroides fragilis (20)	ER-35786	0.20–0.78	0.39	0.39
	Imipenem	0.05–0.20	0.10	0.20
	Meropenem	0.10–0.39	0.20	0.39
	BO-2727	0.39–0.78	0.78	0.78
	Cefclidin	12.5–>100	25	>100
	Ceftazidime	6.25–>100	12.5	>100

μg/ml, as the reference carbapenems, and was 64 times more active than cefclidin and ceftazidime.

Among the anaerobes, ER-35786 exhibited high activity against *Peptostreptococcus* spp. and *Bacteroides fragilis* (MIC_{90}s, 0.20 and 0.39 μg/ml, respectively), as did the reference carbapenems, and it was ≥32 times more active than cefclidin and ceftazidime. Three of 20 strains of *B. fragilis* were highly resistant to cephalosporins, yet they were susceptible to carbapenems, including ER-35786.

The MBCs of ER-35786 in Mueller-Hinton broth were identical to or at most twice as high as the MICs for six strains each of methicillin-susceptible *S. aureus*, methicillin-resistant *S. au-*

TABLE 2. Antibacterial activity of ER-35786 against β-lactamase-producing strains

Organism	β-Lactamase		MIC (μg/ml)					
	Classification[a]	Type[b]	ER-35786	Imipenem	Meropenem	BO-2727	Cefclidin	Ceftazidime
Staphylococcus aureus 15009(pI258)	2a (PCase)	C	0.025	≦0.006	0.05	0.025	3.13	3.13
Escherichia coli W3630(Rms212)	2b (PCase)	C	0.05	0.20	0.025	0.10	0.10	0.39
Escherichia coli W3630(Rms213)	2d (PCase)	C	0.05	0.10	0.025	0.10	0.39	0.20
Escherichia coli ML4905(pMS160)	2c (PCase)	C	0.05	0.10	0.025	0.05	0.20	0.39
Klebsiella pneumoniae GN69	2b (PCase)	C	0.025	0.20	0.012	0.05	0.20	0.39
Escherichia coli 255	1 (CSase)	C	0.025	0.10	0.012	0.05	0.10	12.5
Escherichia coli 255/L-7	1 (CSase)	ND	0.05	0.10	0.025	0.05	0.05	0.20
Enterobacter cloacae E10045 SKR5	1 (CSase)	DR	0.05	0.20	0.05	0.10	0.39	100
Enterobacter cloacae E10045 WT	1 (CSase)	I	0.05	0.39	0.05	0.10	0.10	0.39
Serratia marcescens GN10857	1 (CSase)	I	0.39	0.78	0.10	0.39	1.56	1.56
Morganella morganii 1510	1 (CSase)	DR	0.78	3.13	0.10	1.56	0.05	6.25
Morganella morganii 1510/9	1 (CSase)	ND	0.39	3.13	0.10	1.56	0.05	0.05
Providencia rettgeri GN4430	1 (CSase)	I	0.20	0.39	0.05	0.39	0.05	3.13
Pseudomonas aeruginosa E03441 SKR2	1 (CSase)	DR	0.20	1.56	0.78	0.78	3.13	50
Pseudomonas aeruginosa E03441 WT	1 (CSase)	I	0.20	1.56	0.39	0.39	0.39	3.13
Klebsiella oxytoca E23073	2be (CXase)	I	0.05	0.20	0.025	0.10	0.78	0.39
Proteus vulgaris GN7919	2e (CXase)	I	0.39	0.78	0.05	0.78	0.78	0.10
Stenotrophomonas maltophilia GN12873	2e, 3 (CXase)	I	>100	>100	100	>100	12.5	100
Bacteroides fragilis E51035	2e (CXase)	I	0.39	0.10	0.20	0.78	25	12.5

[a] Bush-Jacoby-Medeiros classification (2); the Mitsuhashi-Inoue classification (11) is provided in parentheses. PCase, penicillinase; CSase, cephalosporinase; CXase, oxyiminocephalosporinase.
[b] Expression of enzyme production: C, constitutive; DR, derepressed; I, inducible; ND, not detected.

reus, *E. coli*, *S. marcescens*, and *P. aeruginosa*. The reference compounds also showed bactericidal effects (MBCs/MICs) similar to those of ER-35786 (data not shown).

Resistance to hydrolysis by β-lactamases. The susceptibility of ER-35786 to enzymatic hydrolysis was tested with various types of β-lactamases. Hydrolysis of ER-35786 was expressed relative to the absolute rate of cephaloridine or penicillin G hydrolysis, taken as 100. ER-35786 was highly resistant to hydrolysis by all group 1 β-lactamases (2) (cephalosporinase [11] tested from *E. cloacae*, *C. freundii*, *P. rettgeri*, and *P. aeruginosa*, as were imipenem and meropenem (relative hydrolysis, <0.5%). ER-35786 was also highly resistant to group 2b, 2d, and 2c β-lactamases (2) (penicillinase types I, II, and IV, respectively [11] (relative hydrolysis, <1%). ER-35786 was hydrolyzed to a slight extent (relative hydrolysis, 3.4%) by the group 2b β-lactamase produced by *K. pneumoniae* GN69, as were imipenem and meropenem. ER-35786 was as stable against the group 2e β-lactamase (2) (oxyiminocephalosporinase [11] produced by *B. fragilis* E51036 as imipenem and meropenem. However, ER-35786, imipenem, and meropenem were efficiently hydrolyzed by the group 3 β-lactamase (2) (oxyiminocephalosporinase) produced by *S. maltophilia* GN12873, with relative rates of hydrolysis of 38.1, 55.6, and 25.0%, respectively. The susceptibility profile of ER-35786 to β-lactamases was almost the same as those of imipenem and meropenem.

The activities of ER-35786 against strains producing various types of β-lactamases are presented in Table 2. The group 2a, 2b, 2c, and 2d β-lactamase (penicillinase)-producing bacteria were susceptible to all the carbapenems tested. The activities of carbapenems, including ER-35786, against the group 1 β-lactamase (cephalosporinase)-overproducing strains were little affected by the derepressed production of β-lactamase in comparison with those against the strains which produced low levels of β-lactamase, whereas the activity of ceftazidime was greatly reduced by the derepressed production of β-lactamase. Most of the group 2b, 2be, and 2e β-lactamase (oxyiminocephalosporinase)-producing strains were inhibited by ER-35786 at a concentration of ≤0.39 μg/ml, as was the case with the other carbapenems. *S. maltophilia* GN12873, which produced both group 2e and 3 β-lactamases, was resistant to all the compounds tested.

Features of antipseudomonal activity of ER-35786. The activity of ER-35786 was measured against β-lactam-resistant strains of *P. aeruginosa*, with mutations in chromosomal group 1 β-lactamase production or in expression of the outer membrane proteins, which were derived from a clinical isolate, E03441 WT (Table 3). Irrespective of the presence of OprD, increasing levels of β-lactamase had the strongest effect on activities of cephalosporins, which were lowered to 1/8 to 1/32, and the OprD little affected cephalosporin activities. In the presence of OprD (strains SKR2-14, WT, and SKR2), the β-lactamase level had much less of an effect on carbapenems: two times less for ER-35786 and four times less for the other compounds. In the absence of OprD (strains SKR2/I-2, R24, and SKR2/I), increasing β-lactamase levels led to resistance to all the carbapenems (4 to 32 times). Interestingly, in the presence of low-level β-lactamase activity, a lack of OprD reduced the imipenem activity by only twofold, whereas the meropenem activity was reduced to 1/16. This result is consistent with that of Livermore (7).

Overexpression of an outer membrane protein, OprM, was reported in meropenem-resistant mutants and ofloxacin-cefsulodin-resistant mutants of *P. aeruginosa* (9). It resulted in resistance to various antibiotics, meropenem, cephalosporins, quinolones, tetracycline, and chloramphenicol, probably due to its role as an efflux pump (9). The amount of an outer membrane protein with a molecular size of 48 kDa (p48) was increased in strains E03441 MR-3 and E03441 OCR-5 isolated in this study, and this protein is likely to be OprM. In addition, the OprD protein was absent from strain E03441 MR-3, selected for meropenem resistance, as reported previously (9). In the presence of β-lactamase expression, the production of p48 protein affected the activities of meropenem, cefclidin, and ceftazidime, and their activities against strain E03441 OCR-5 were reduced to one-fourth of those against strain E03441 WT. In contrast, the activity of ER-35786 was not reduced, like those of imipenem and BO-2727.

TABLE 3. Activity of ER-35786 against *P. aeruginosa* mutants resistant to β-lactams

Strain	Origin	β-Lactamase specific activity (nmol/min/mg of proteins)[a]		Presence of OprD	Presence of p48[c]	MIC (μg/ml)					
		Uninduced	Induced[b]			ER-35786	Imipenem	Meropenem	BO-2727	Cefclidin	Ceftazidime
E03441 SKR2-14	E03441 SKR2	<0.4	0.8	+	−	0.10	0.39	0.20	0.20	0.39	1.56
E03441 SKR2/I-2	E03441 SKR2/I	4.6	5.2	−	−	0.39	0.78	3.13	0.78	0.39	1.56
E03441 WT		1.3	573.6	+	−	0.20	1.56	0.39	0.39	0.39	1.56
E03441 R24	E03441 WT	2.0	136.2	−	−	3.13	25	12.5	6.25	0.78	3.13
E03441 SKR2	E03441 WT	1,978.8	2,973.8	+	−	0.20	1.56	0.78	0.78	3.13	50
E03441 SKR2/I	E03441 SKR2	2,096.3	2,360.6	−	−	3.13	25	12.5	12.5	3.13	50
E03441 MR-3	E03441 WT	0.5	159.1	−	+	3.13	25	25	6.25	1.56	6.25
E03441 OCR-5	E03441 WT	1.1	647.8	+	+	0.20	1.56	1.56	0.39	1.56	6.25

[a] β-Lactamase activity was measured spectrophotometrically by using cephalothin as the substrate.
[b] Cells grown for 2 h in the presence of 0.10 μg of imipenem per ml.
[c] Outer membrane protein with a molecular size of 48 kDa.

Activity against systemic infections in mice. The protective efficacy of ER-35786 against experimental systemic infections caused by *S. aureus*, *E. coli*, *K. pneumoniae*, *S. marcescens*, and *P. aeruginosa* in mice was compared with those of imipenem-cilastatin, meropenem, BO-2727, and cefclidin (Table 4). In the case of *S. aureus* infection, cefluprenam was tested instead of cefclidin. Against infection caused by methicillin-susceptible *S. aureus* E31290, ER-35786 was nine times more effective than meropenem and was as effective as BO-2727, although it was less effective than imipenem-cilastatin, with an ED_{50} of 0.112 mg/kg. Against methicillin-resistant *S. aureus* E31404 infection, ER-35786 was as effective as BO-2727 and was more effective than imipenem-cilastatin or meropenem, with an ED_{50} of 14.3 mg/kg. For infections caused by *E. coli* E01125Y, *K. pneumoniae* E02264, and *S. marcescens* E11272, the ED_{50}s of ER-35786 were 0.0980, 0.0325, and 1.28 mg/kg, respectively, and were comparable to those of meropenem and cefclidin, two to four times lower than those of BO-2727, and four to eight times lower than those of imipenem-cilastatin. Against *P. aeruginosa* infections, ER-35786 showed the highest efficacy among the compounds tested. Against infection caused by *P. aeruginosa* E03763, the in vivo activity of ER-35786 was 2.5 times higher than those of imipenem-cilastatin and BO-2727, with an ED_{50} of 0.264 mg/kg. Against imipenem-resistant *P. aeruginosa* E03763 IR, ER-35786 was as active as imipenem and cefclidin and was more active than meropenem and BO-2727, with an ED_{50} of 4.02 mg/kg. Against cefclidin-resistant *P. aeruginosa* E03402, ER-35786 was the most active among the compounds tested, with an ED_{50} of ≤0.313 mg/kg. Against *P. aeruginosa* E03402 CRIR, which is resistant to cefclidin, ceftazidime, and imipenem, ER-35786 was again the most effective compound, with an ED_{50} of 2.71 mg/kg.

Activity against acute pneumonia caused by *P. aeruginosa*. A discrepancy between the in vitro activity and the in vivo activity of carbapenems and cefclidin was seen when the mice used for the systemic model of infection caused by *P. aeruginosa* were treated with a single dose of each compound (Table 4). The higher in vivo activity of carbapenems may result from the higher bactericidal activity of carbapenems than that of cefclidin over a short period and the shorter elimination $t_{1/2}$s of compounds in mice than in humans. Therefore, in the acute pneumonia model, in which the rate of reduction of the viable cell count in the lungs was examined, the compounds had to be administered in multiple doses to maintain effective concentrations in immunosuppressed mice treated with 5-FU.

In acute pneumonia caused by *P. aeruginosa* E03763, ER-35786 was more effective in reducing the numbers of CFU in the lungs than imipenem-cilastatin, meropenem, and BO-2727 ($P < 0.01$). Against imipenem-resistant *P. aeruginosa* E03763 IR, ER-35786 was also more effective in reducing the numbers of CFU in the lungs than imipenem-cilastatin ($P < 0.05$), meropenem ($P < 0.01$), or BO-2727 ($P < 0.01$). Against cefclidin-resistant *P. aeruginosa* E03402, ER-35786 was the most effective among the compounds tested ($P < 0.01$). Against cefclidin-ceftazidime-imipenem-resistant *P. aeruginosa* E03402 CRIR, ER-35786 was also the most effective among the compounds tested ($P < 0.01$) (Table 5).

Susceptibility to hydrolysis by renal DHP-Is. The susceptibility of ER-35786 to enzymatic hydrolysis was tested with renal DHP-Is from mice, swine, and humans (Table 6). ER-35786 was about three to five times more stable against mouse renal DHP-I than imipenem and meropenem, as was BO-2727. Against swine renal DHP-I, ER-35786 and BO-2727 were as stable as meropenem, and against human renal DHP-I, ER-35786 and BO-2727 were about two times more stable than meropenem. Imipenem was hydrolyzed by swine and human renal DHP-Is about five times more rapidly than meropenem was. The profile of the susceptibility of ER-35786 to renal DHP-Is was similar to that of BO-2727.

DISCUSSION

ER-35786 is a new parenteral 1β-methyl carbapenem with a broad-spectrum, potent antibacterial activity against gram-positive and gram-negative aerobic and anaerobic bacteria.

In this study, ER-35786 showed high in vitro activity against gram-positive cocci such as methicillin-susceptible *S. aureus*, streptococci, and enterococci, and this activity could be comparable to those of reference carbapenems such as imipenem and meropenem. The activity of ER-35786 against methicillin-resistant *S. aureus* was also considerably higher than those of the reference compounds. However, methicillin-resistant *S. aureus* is suggested to be intrinsically resistant to ER-35786 as well, since the MIC_{90} and ED_{50} of ER-35786 were about 100 times higher for methicillin-resistant *S. aureus* than for methicillin-susceptible *S. aureus.*

ER-35786 was also highly active against the members of the family *Enterobacteriaceae*, *M. catarrhalis*, and *H. influenzae*, although the activity of ER-35786 was slightly lower than that of meropenem. No cross-resistance between ER-35786 and the cephalosporins tested was observed with ceftazidime-resistant strains of *E. cloacae*, *C. freundii*, and *M. morganii*. Since resistance in these strains is mainly due to hydrolysis of compounds by the overproduced group 1 β-lactamases (18), ER-35786 is suggested to be highly resistant to hydrolysis by group 1 β-lactamases. However, 3 of 56 clinical isolates of *S. marcescens*

TABLE 4. Comparative activities of ER-35786 and reference compounds against systemic infections in mice

Organism	Infective dose (CFU/mouse)	Compound	MIC (μg/ml)	ED_{50} (mg/kg) (95% confidence limits)
Staphylococcus aureus E31290, methicillin susceptible	2.3×10^6 (>10× LD_{50}[a])	ER-35786	0.05	0.112 (0.0880–0.142)
		Imipenem-cilastatin	0.025	0.0317 (0.0222–0.0452)
		Meropenem	0.10	1.02 (0.734–1.43)
		BO-2727	0.10	0.0867 (0.0777–0.628)
		Cefluprenam	0.39	0.539 (0.463–0.628)
Staphylococcus aureus E31404, methicillin resistant	1.8×10^7 (>10× LD_{50})	ER-35786	12.5	14.3 (11.1–18.5)
		Imipenem-cilastatin	100	>20
		Meropenem	50	>20
		BO-2727	25	16.2 (11.9–22.0)
		Cefluprenam	100	28.1 (19.1–41.2)
Escherichia coli E01125Y	7.7×10^3 (>30× LD_{50})	ER-35786	0.025	0.0980 (0.0619–0.155)
		Imipenem-cilastatin	0.20	0.884 (0.707–1.11)
		Meropenem	0.025	0.191 (0.117–0.311)
		BO-2727	0.10	0.340 (0.246–0.471)
		Cefclidin	0.05	0.116 (0.0885–0.152)
Klebsiella pneumoniae E02264	6.3×10^3 (>10× LD_{50})	ER-35786	0.05	0.0325 (0.0228–0.0464)
		Imipenem-cilastatin	0.20	0.135 (0.0837–0.216)
		Meropenem	0.05	0.0251 (0.0167–0.0379)
		BO-2727	0.10	0.111 (0.0830–0.149)
		Cefclidin	0.05	0.0208 (0.0125–0.0347)
Serratia marcescens E11272	1.5×10^6 (>10× LD_{50})	ER-35786	0.39	1.28 (0.812–2.01)
		Imipenem-cilastatin	0.78	7.27 (4.75–11.1)
		Meropenem	0.10	1.13 (0.616–2.07)
		BO-2727	0.78	2.66 (1.62–4.38)
		Cefclidin	0.39	0.951 (0.506–1.79)
Pseudomonas aeruginosa E03763	1.23×10^4 (>30× LD_{50})	ER-35786	0.39	0.264 (0.186–0.374)
		Imipenem-cilastatin	3.13	0.693 (0.526–0.912)
		Meropenem	0.78	4.70 (3.87–5.70)
		BO-2727	1.56	0.614 (0.505–0.745)
		Cefclidin	0.78	12.6 (8.75–18.3)
Pseudomonas aeruginosa E03763IR, imipenem resistant	1.60×10^4 (8.56× LD_{50})	ER-35786	3.13	4.02 (3.05–5.29)
		Imipenem-cilastatin	25	6.62 (5.03–8.71)
		Meropenem	6.25	>10
		BO-2727	6.25	>10
		Cefclidin	0.78	8.67 (5.23–14.4)
Pseudomonas aeruginosa E03402, cefclidin resistant	1.83×10^5 (42.7× LD_{50})	ER-35786	0.20	<0.313
		Imipenem-cilastatin	1.56	0.791 (0.584–1.07)
		Meropenem	0.39	2.54 (1.53–4.22)
		BO-2727	0.39	0.658 (0.423–1.02)
		Cefclidin	25	>20
Pseudomonas aeruginosa E03402 CRIR, cefclidin, ceftazidime, and imipenem resistant	4.50×10^5 (5.47× LD_{50})	ER-35786	3.13	2.71 (1.73–4.24)
		Imipenem-cilastatin	25	4.75 (3.06–7.39)
		Meropenem	25	>10
		BO-2727	6.25	>10
		Cefclidin	25	>20

[a] LD_{50}, 50% lethal dose.

tested were resistant to carbapenems, including ER-35786. Recently, newer β-lactamases hydrolyzing imipenem have been identified from clinical isolates of *S. marcescens* (8, 15). The carbapenem-resistant *S. marcescens* strain used in this study might produce carbapenem-hydrolyzing β-lactamase. Furthermore, 7 of 39 clinical isolates of *H. influenzae* were resistant to imipenem, yet the mechanism of this resistance is unknown.

One of the most important features of ER-35786 is its potent effectiveness against *P. aeruginosa*. ER-35786 showed at least two times higher activity against *P. aeruginosa* than the reference compounds. ER-35786 was especially active against imipenem- or meropenem-resistant strains of *P. aeruginosa*, although cross-resistance between ER-35786 and other carbapenems was observed. The main permeation route of basic carbapenems through the outer membrane of *P. aeruginosa* is suggested to be the OprD porin. However, the permeation of ER-35786, which has a molecular weight of 411.5, is likely to be less efficient than that of imipenem, which has a molecular

TABLE 5. Effect of ER-35786 and other compounds on acute pneumonia model in mice[a]

Organism	Dose (mg/kg)	Compound	Log CFU/lung
Pseudomonas aeruginosa E03763	0	Control	4.44 ± 0.50
	0.156	ER-35786	2.67 ± 0.35**[b]
		Imipenem-cilastatin	4.50 ± 0.42‡[c]
		Meropenem	4.23 ± 0.49‡
		BO-2727	4.17 ± 0.24‡
		Cefclidin	2.85 ± 0.47**
	0.313	ER-35786	2.14 ± 0.23**
		Imipenem-cilastatin	3.67 ± 0.32‡
		Meropenem	3.41 ± 0.67*,‡
		BO-2727	2.97 ± 0.72**
		Cefclidin	2.39 ± 0.48**
Pseudomonas aeruginosa E03763IR	0	Control	3.80 ± 0.35
	2.5	ER-35786	2.75 ± 0.67**
		Imipenem-cilastatin	2.54 ± 0.32**
		Meropenem	3.95 ± 0.40‡
		BO-2727	3.14 ± 0.53
		Cefclidin	1.69 ± 0.21**
	5	ER-35786	1.55 ± 0.23**
		Imipenem-cilastatin	2.46 ± 0.74**,†
		Meropenem	3.59 ± 0.35‡
		BO-2727	2.58 ± 0.07**,‡
		Cefclidin	1.49 ± 0.27**
Pseudomonas aeruginosa E03402	0	Control	5.24 ± 0.27
	0.078	ER-35786	2.50 ± 0.34**
		Imipenem-cilastatin	5.23 ± 0.46‡
		Meropenem	4.98 ± 0.26‡
		BO-2727	4.89 ± 0.26‡
		Cefclidin	5.26 ± 0.27‡
	0.156	ER-35786	2.44 ± 0.21**
		Imipenem-cilastatin	4.84 ± 0.46‡
		Meropenem	4.69 ± 0.32‡
		BO-2727	4.89 ± 0.26**,‡
		Cefclidin	5.26 ± 0.27‡
Pseudomonas aeruginosa E03402CRIR	0	Control	5.43 ± 0.31
	2.5	ER-35786	3.00 ± 0.66**
		Imipenem-cilastatin	4.85 ± 0.14‡
		Meropenem	5.54 ± 0.16‡
		BO-2727	5.05 ± 0.33‡
		Cefclidin	5.25 ± 0.25‡
	5	ER-35786	2.27 ± 0.18**
		Imipenem-cilastatin	3.04 ± 0.46**
		Meropenem	5.05 ± 0.27‡
		BO-2727	3.53 ± 0.70**,‡
		Cefclidin	4.59 ± 0.37*,‡

[a] The inocula were 6.0×10^3 CFU of E03763 per mouse, 3.2×10^3 CFU of E03763IR per mouse, 6.3×10^4 CFU of E03402 per mouse, and 8.3×10^4 of E03402 CRIR per mouse.
[b] Comparison with control group by Dunnett's test; **, $P < 0.01$; *, $P < 0.05$.
[c] Comparison with ER-35786 group by the Tukey-Kramer honestly significant difference test: ‡, $P < 0.01$; †, $P < 0.05$.

weight of 299, since the permeation rates of basic carbapenem or penem compounds through OprD are correlated to molecular weight (23). Therefore, the high antipseudomonal activity of ER-35786 may result mainly from its high affinity for certain lethal penicillin-binding proteins or for primary penicillin-binding protein targets different from those of other carbapenems. Indeed, the morphological changes of *P. aeruginosa* cells treated with ER-35786 were slightly different from those of cells treated with imipenem and meropenem: spindle forms and bulging forms were observed in the case of ER-35786,

TABLE 6. Susceptibility of ER-35786 to hydrolysis by renal DHP-Is

Compound	Relative $t_{1/2}$ of hydrolysis by renal DHP-I from[a]:		
	Mouse	Swine	Human
ER-35786	5.00	1.24	1.96
Imipenem	1.52	0.16	0.19
Meropenem	1.00[b]	1.00[c]	1.00[d]
BO-2727	4.96	1.38	2.18

[a] With the $t_{1/2}$ of meropenem hydrolysis taken as 1.
[b] 0.81 h.
[c] 2.66 h.
[d] 5.57 h.

while ovoid forms were observed in cells treated with imipenem and filamented forms were observed in those treated with meropenem (data not shown).

Imipenem resistance in *P. aeruginosa* might mainly be due to the reduction or lack of OprD (1, 22), which also deteriorated the antipseudomonal activity of ER-35786. However, the imipenem MIC for the strain deficient in the production of β-lactamase and OprD was only two times higher than that for the strain deficient in the production of β-lactamase. On the other hand, the meropenem MIC was increased 16 times. The MICs of carbapenems for the strain deficient in OprD but producing β-lactamase was 16 to 32 times higher than those for the strain producing β-lactamase. Therefore, the resistance of carbapenems in *P. aeruginosa* could result from an interplay of β-lactamase hydrolysis and impermeability, but not either factor alone. Moreover, it is noteworthy that ER-35786 activity, like that of imipenem, was little affected by the production of OprM. Further studies on the high antipseudomonal activity of ER-35786 are needed.

ER-35786 was more resistant than meropenem to hydrolysis by renal DHP-I from mice, and urinary recovery of ER-35786 (given intravenously) at 10 mg/kg was 50.4% in mice within 6 h (5). Indeed, coadministration of cilastatin had little effect on the in vivo activity of ER-35786 against a systemic infection model (14). The binding rates of ER-35786 to proteins in the sera of various animals were also very low (5). The effectiveness of ER-35786 against various *P. aeruginosa* infection models is considered to result from its high antipseudomonal activity coupled with its high level of resistance to DHP-I hydrolysis and its low protein-binding rates. It is suggested that ER-35786 would not require the coadministration of an inhibitor in clinical use, since ER-35786 was more stable than meropenem and imipenem against human renal DHP-I.

In conclusion, ER-35786 is a promising new parenteral carbapenem for the treatment of opportunistic and nosocomial infections, especially those involving *P. aeruginosa* isolates resistant to various antibiotics. Further studies on its activity, as well as the pharmacokinetic and toxicological characteristics, are warranted.

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