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In Vitro and In Vivo Antibacterial Activities of SM-216601, a New Broad-Spectrum Parenteral Carbapenem

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SM-216601 is a novel parenteral 1β-methylcarbapenem. In agar dilution susceptibility testing, the MIC of SM-216601 for 90% of the methicillin-resistant *Staphylococcus aureus* (MRSA) strains tested (MIC_{90}) was 2 μg/ml, which was comparable to those of vancomycin and linezolid. SM-216601 was also very potent against *Enterococcus faecium*, including vancomycin-resistant strains (MIC_{90} = 8 μg/ml). SM-216601 exhibited potent activity against penicillin-resistant *Streptococcus pneumoniae*, ampicillin-resistant *Haemophilus influenzae*, *Moraxella catarrhalis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*, with MIC_{90}s of less than 0.5 μg/ml, and intermediate activity against *Citrobacter freundii*, *Enterobacter cloacae*, *Serratia marcescens*, and *Pseudomonas aeruginosa*. The therapeutic efficacy of SM-216601 against experimentally induced infections in mice caused by *S. aureus*, *E. faecium*, *E. coli*, and *P. aeruginosa* reflected its in vitro activity and plasma level. Thus, SM-216601 is a promising candidate for nosocomial bacterial infections caused by a wide range of gram-positive and gram-negative bacteria, including multiresistant pathogens.

The emergence of multiresistant gram-positive cocci such as methicillin-resistant staphylococci, vancomycin-resistant enterococci, and penicillin-resistant *Streptococcus pneumoniae* (PRSP) has reduced the value of antibacterial chemotherapy in recent years (3, 16). Resistance to β-lactam antibiotics is a particularly serious concern because of the advantages of β-lactams over other classes of antibacterials. A broad antibacterial spectrum, bactericidal activity, and an excellent safety profile make them suitable for empirical treatment of a variety of bacterial infections, especially for pediatric and immunocompromised patients in hospitals. Therefore, there is an urgent need for new β-lactams effective against resistant pathogens.

We previously reported that a 2-(4-arylthiazol-2-ylthio)-1β-methylcarbapenem, SM-17466, and its derivatives exhibited potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA) corresponding to a high affinity for bacterial penicillin-binding protein 2a (PBP2a) (22, 28). SM-17466 showed a higher level of activity against *Enterococcus faecium* than existing carbapenems, prompting us to search for new carbapenems with potent activity against MRSA and vancomycin-resistant *E. faecium* (VRE) (25). Consequently, we identified SM-197436, SM-232721, and SM-232724, having a dihydropyrrole or tetrahydropyridine ring instead of the pyridium moiety of SM-17466 at the C-2 side chain, with improved activity against *E. faecium* (26). In addition, our observation that the dihydropyrrole derivative SM-197436 was more active against gram-negative bacteria than SM-232721 and SM-232724 suggested the possibility of developing novel broad-spectrum carbapenems (34). We examined the structure-activity relationships of a series of 2-(4-tetrahydropyridinylthiazol-2-ylthio)-1β-methylcarbapenems and 4-dihydropyrrolyl thiazole analogs with regard to their antibacterial activities especially against gram-negative bacteria and their convulsant activity in mice and selected SM-216601 as a promising candidate for a broad-spectrum carbapenem (Fig. 1) (33).

In this study, we investigated the activity of SM-216601 against various clinical isolates in comparison with vancomycin, linezolid, and several other β-lactam antibiotics. A time-kill study of SM-216601 for key resistant pathogens, such as MRSA, VRE, PRSP, ampicillin-resistant *Haemophilus influenzae*, and *Pseudomonas aeruginosa* was also done. In addition, the efficacy of SM-216601 against systemic infections caused by methicillin-susceptible *S. aureus* (MSSA), MRSA, *Escherichia coli*, and *P. aeruginosa* and experimental *E. faecium* subcutaneous abscesses was evaluated in mice.

(Part of this work was presented in abstract form at the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy, 30 October to 2 November 2004, Washington, D.C. [abstr. F-330, p. 197].)

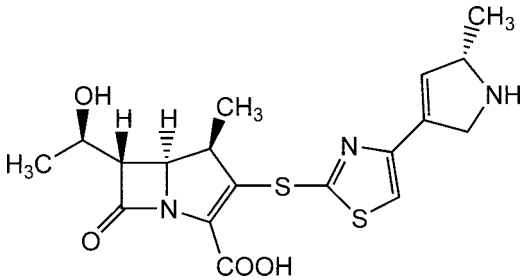

FIG. 1. Chemical structure of SM-216601.

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TABLE 1. Antibacterial activities of SM-216601 against clinical isolates

Organism (no. of isolates) and antimicrobial agent	MIC (μg/ml)		
	Range	MIC_{50}	MIC_{90}
MSSA (12)			
SM-216601	≤0.016–0.031	≤0.016	≤0.016
Imipenem	≤0.016–0.031	≤0.016	0.031
Panipenem	0.063	0.031	0.031
Meropenem	0.063–0.125	0.125	0.125
Flomoxef	0.5	0.5	0.5
Oxacillin	0.125–0.5	0.25	0.25
Vancomycin	0.5–1	0.5	1
Linezolid	2	2	2
MRSA (30)			
SM-216601	0.063–2	1	2
Imipenem	≤0.063–64	32	64
Panipenem	0.125–32	16	32
Meropenem	0.5–32	16	32
Flomoxef	4–128	64	64
Oxacillin	4–>32	>32	>32
Vancomycin	0.25–1	1	1
Linezolid	1–2	1	2
Methicillin-susceptible *Staphylococcus epidermidis* (13)			
SM-216601	≤0.016	≤0.016	≤0.016
Imipenem	≤0.016	≤0.016	≤0.016
Panipenem	≤0.016–0.31	0.031	0.031
Meropenem	0.063–0.125	0.063	0.125
Flomoxef	0.25–1	0.5	1
Oxacillin	0.125–0.25	0.125	0.25
Vancomycin	0.5–1	1	1
Linezolid	0.5–1	1	1
Methicillin-resistant *Staphylococcus epidermidis* (12)			
SM-216601	0.125–1	0.25	0.5
Imipenem	0.125–32	0.25	16
Panipenem	0.25–16	0.5	16
Meropenem	1–16	2	16
Flomoxef	2–16	4	16
Oxacillin	2–>32	4	>32
Vancomycin	1	1	1
Linezolid	0.5–1	1	1
Streptococcus pyogenes (12)			
SM-216601	≤0.016	≤0.016	≤0.016
Imipenem	≤0.016	≤0.016	≤0.016
Panipenem	≤0.016	≤0.016	≤0.016
Meropenem	≤0.016	≤0.016	≤0.016
Flomoxef	0.125–0.25	0.25	0.25
Vancomycin	0.5	0.5	0.5
Linezolid	1–2	2	2
Streptococcus agalactiae (12)			
SM-216601	≤0.016	≤0.016	≤0.016
Imipenem	≤0.016–0.031	≤0.016	≤0.016
Panipenem	≤0.016–0.031	≤0.016	0.031
Meropenem	0.031–0.063	0.063	0.063
Flomoxef	0.25–1	0.5	0.5
Vancomycin	0.5	0.5	0.5
Linezolid	1–2	2	2
Penicillin-susceptible *Streptococcus pneumoniae* (12)			
SM-216601	≤0.016	≤0.016	≤0.016
Imipenem	≤0.016	≤0.016	≤0.016
Panipenem	≤0.016	≤0.016	≤0.016
Meropenem	≤0.016–0.031	≤0.016	0.031
Flomoxef	0.125–0.25	0.25	0.25
Penicillin G	≤0.016–0.063	0.063	0.063
Vancomycin	0.125–0.5	0.5	0.5
Linezolid	0.5–2	1	2

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TABLE 1—*Continued*

Organism (no. of isolates) and antimicrobial agent	MIC (μg/ml)		
	Range	MIC_{50}	MIC_{90}
Penicillin-intermediate *Streptococcus pneumoniae* (16)			
SM-216601	≤0.016–0.125	0.031	0.125
Imipenem	≤0.016–0.25	0.063	0.125
Panipenem	≤0.016–0.125	0.063	0.125
Meropenem	0.031–0.5	0.25	0.5
Flomoxef	0.25–4	1	4
Penicillin G	0.125–1	0.5	1
Vancomycin	0.25–0.5	0.5	0.5
Linezolid	0.5–2	1	1
PRSP (14)			
SM-216601	0.063–0.25	0.125	0.25
Imipenem	0.125–0.25	0.25	0.25
Panipenem	0.063–0.125	0.125	0.125
Meropenem	0.25–0.5	0.5	0.5
Flomoxef	2–8	4	8
Penicillin G	2–4	2	4
Vancomycin	0.25–0.5	0.5	0.5
Linezolid	1	1	1
Enterococcus faecalis (12)			
SM-216601	1–2	2	2
Imipenem	1–2	2	2
Panipenem	1–4	2	4
Meropenem	8–16	8	8
Flomoxef	>32	>32	>32
Ampicillin	1–2	2	2
Vancomycin	1	1	1
Linezolid	2	2	2
Enterococcus faecium (32)[a]			
SM-216601	0.5–16	4	8
Imipenem	8–>128	128	>128
Panipenem	8–>128	>128	>128
Meropenem	32–>128	>128	>128
Flomoxef	64–>128	>128	>128
Ampicillin	4–>128	64	>128
Vancomycin	0.5–>32	>32	>32
Linezolid	2	2	2
Peptostreptococcus spp. (12)[b]			
SM-216601	≤0.016–1	0.063	0.5
Imipenem	≤0.016–2	0.125	0.5
Panipenem	≤0.016–2	0.125	1
Meropenem	≤0.016–2	0.125	0.5
Flomoxef	≤0.063–8	0.25	8
Ampicillin-susceptible *Haemophilus influenzae* (15)			
SM-216601	0.063–0.125	0.063	0.125
Imipenem	2–32	4	16
Panipenem	0.25–16	2	16
Meropenem	0.063–0.5	0.125	0.125
Flomoxef	1–16	1	16
Ampicillin	0.25–2	0.5	1
Ampicillin-resistant *Haemophilus influenzae* (15)			
SM-216601	0.031–0.25	0.125	0.25
Imipenem	1–64	8	32
Panipenem	1–32	4	32
Meropenem	0.031–0.5	0.5	0.5
Flomoxef	1–>32	16	>32
Ampicillin	4–>128	128	>128
Moraxella catarrhalis (12)			
SM-216601	≤0.016–0.063	≤0.016	0.031
Imipenem	≤0.016–0.125	0.063	0.125
Panipenem	≤0.016–0.063	0.031	0.063
Meropenem	≤0.016	≤0.016	≤0.016
Flomoxef	0.031–0.5	0.25	0.5

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TABLE 1—*Continued*

Organism (no. of isolates) and antimicrobial agent	MIC (μg/ml)		
	Range	MIC_{50}	MIC_{90}
Escherichia coli (37)			
SM-216601	0.031–1	0.25	0.5
Imipenem	0.063–1	0.125	0.25
Panipenem	0.125–2	0.25	0.25
Meropenem	≤0.016–0.031	≤0.016	≤0.016
Flomoxef	0.031–0.5	0.063	0.125
Klebsiella pneumoniae (38)			
SM-216601	0.063–0.5	0.25	0.25
Imipenem	0.063–1	0.125	0.25
Panipenem	0.063–2	0.25	0.5
Meropenem	≤0.016–0.031	0.031	0.031
Flomoxef	0.031–0.125	0.063	0.063
Proteus mirabilis (12)			
SM-216601	0.063–0.25	0.125	0.25
Imipenem	0.125–2	0.5	1
Panipenem	0.25–1	0.5	1
Meropenem	0.031–0.063	0.031	0.063
Flomoxef	0.125–0.25	0.25	0.25
Serratia marcescens (12)			
SM-216601	1–>128	2	16
Imipenem	0.125–>32	0.25	2
Panipenem	0.125–>32	0.25	8
Meropenem	≤0.016–>32	0.031	2
Flomoxef	0.25–>128	0.5	>128
Enterobacter cloacae (12)			
SM-216601	1–8	2	8
Imipenem	0.125–1	0.25	0.5
Panipenem	0.125–1	0.25	1
Meropenem	≤0.016–0.5	0.031	0.063
Flomoxef	0.25–>128	4	>128
Citrobacter freundii (12)			
SM-216601	0.125–16	2	4
Imipenem	0.125–0.25	0.125	0.25
Panipenem	0.063–0.25	0.125	0.25
Meropenem	≤0.016–0.031	≤0.016	0.031
Flomoxef	≤0.016–16	0.125	4
Pseudomonas aeruginosa (30)			
SM-216601	4–32	16	32
Imipenem	0.25–16	1	8
Panipenem	0.5–32	4	16
Meropenem	≤0.063–16	0.5	4
Flomoxef	64–>128	>128	>128
Bacteroides fragilis (12)			
SM-216601	0.125–128	0.25	16
Imipenem	0.125–32	0.25	4
Panipenem	0.125–>32	0.25	16
Meropenem	0.125–>32	0.25	16
Flomoxef	0.5–>128	2	64

[a] Including 20 strains of VRE.
[b] Including three strains of *P. anaerobius*, two strains of *P. magnus*, two strains of *P. micros*, two strains of *P. prevotii*, and three strains of *P. asaccharolyticus.*

MATERIALS AND METHODS

Organisms. Most of the clinical isolates used in this study were collected from different patients in various hospitals in Japan from 2001 to 2002. Twenty strains of clinical isolates of VRE were obtained from various institutions or hospitals in the United States and Europe. The β-lactamase-producing organisms were from our bacterial collections. All isolates were maintained in glycerol broth at −80°C.

Antimicrobial agents. SM-216601, meropenem, and linezolid were synthesized in the laboratories of the Sumitomo Pharmaceuticals Research Division. Imipenem and cilastatin were prepared from Tienam (Banyu Pharmaceutical Co., Ltd., Tokyo, Japan). Panipenem was prepared from Carbenin (Sankyo Co., Ltd., Tokyo, Japan). Benzyl[^{14}C]penicillin (PCG) was purchased from Amersham International, Plc. (Buckinghamshire, United King-

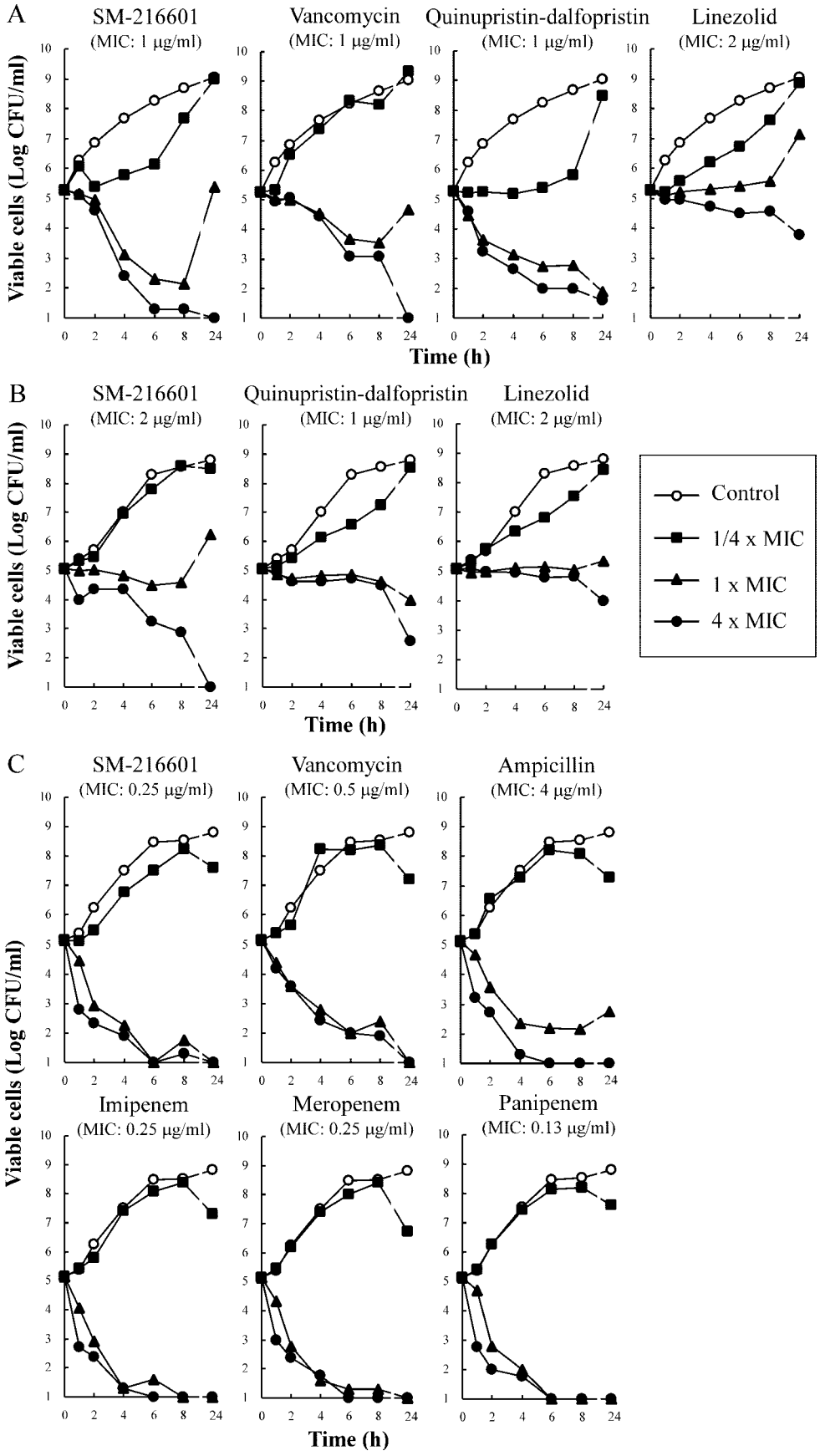

FIG. 2. Bactericidal activities of SM-216601 and reference compounds against (A) *S. aureus* SP-12249 (MRSA), (B) *E. faecium* TL-3273, and (C) *S. pneumoniae* 181 (PRSP).

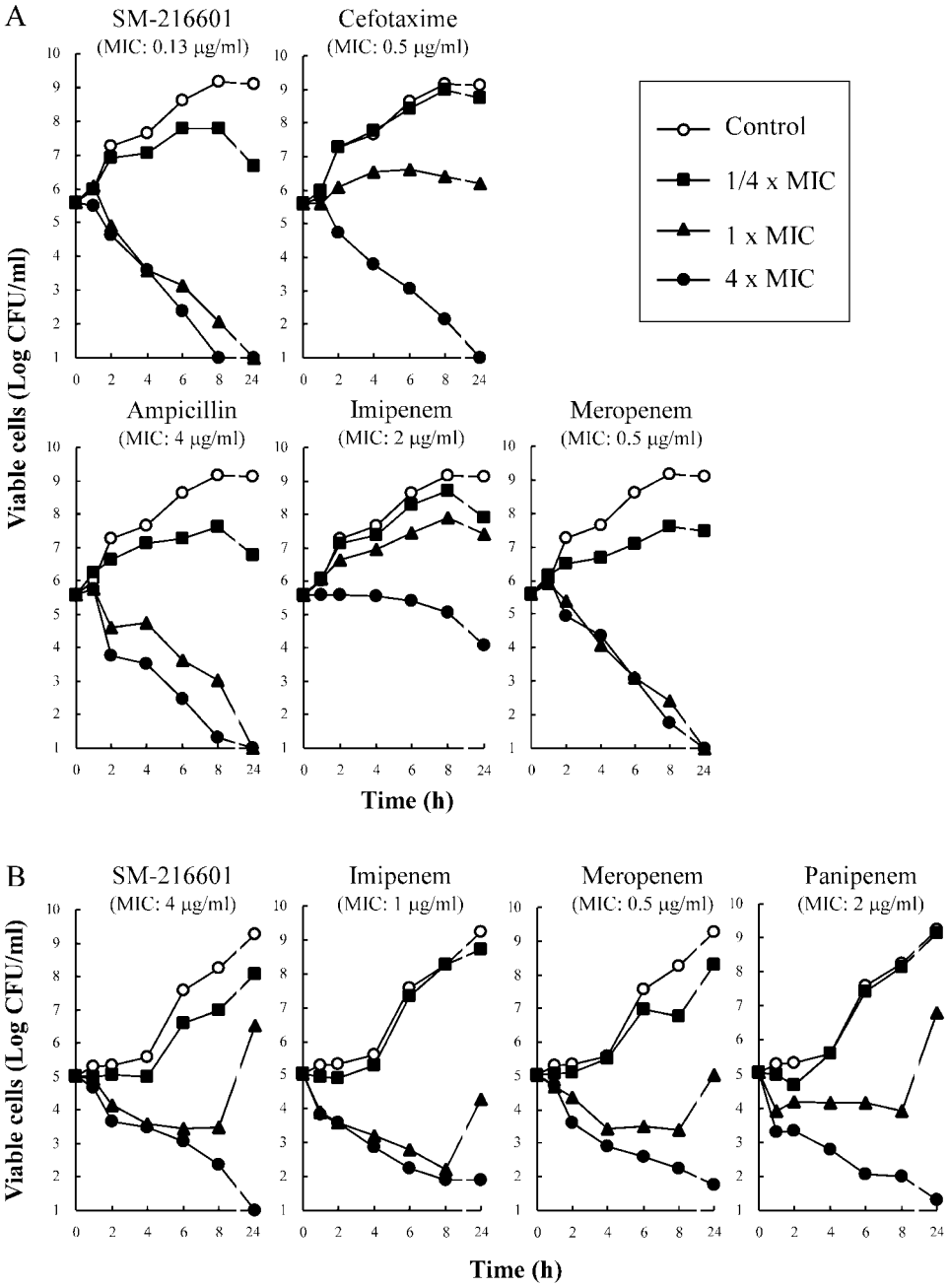

FIG. 3. Bactericidal activities of SM-216601 and reference compounds against (A) *H. influenzae* 231 (β-lactamase negative, ampicillin resistant) and (B) *P. aeruginosa* IFO3451.

dom). The other antimicrobial agents were obtained from commercial sources.

Susceptibility testing. MICs were determined by the twofold serial agar dilution method with Mueller-Hinton agar (MHA; Nippon BD Company Ltd., Tokyo, Japan) unless otherwise specified. Susceptibility testing was performed with MHA supplemented with 5% defibrinated horse blood for streptococci and with 5% Fildes enrichment (BBL Microbiology Systems, Cockeysville, Md.) for *H. influenzae*. Brucella HK agar (Kyokutoh Seiyaku, Tokyo, Japan) supplemented with 5% defibrinated horse blood was used for the culture of anaerobic bacteria. The final inocula comprised approximately 10^4 and 10^6 CFU per spot for aerobic bacteria and anaerobic bacteria, respectively. Agar plates were incubated at 35°C for 18 to 24 h. Incubation was carried out anaerobically in GasPak jars (BBL) for anaerobes and in an atmosphere of 5% CO_2 for streptococci, *H. influenzae*, and *Moraxella catarrhalis*. The MIC was defined as the lowest drug concentration that completely prevented visible growth.

Time-kill assay. The bactericidal activities of the drugs against each of the strains of *S. aureus*, *E. faecium*, *S. pneumoniae*, *H. influenzae*, and *P. aeruginosa* tested were assessed by conducting time-kill assays (21). The test organisms (about 10^5 CFU/ml) were precultured at 35°C for 1 h and consequently treated with the drugs at one-fourth, one, and four times the MIC with shaking at 35°C. Aliquots were removed 1, 2, 4, 6, 8, and 24 h after drug addition. An undiluted aliquot (50 μl) and 10-fold serial dilutions of the aliquot were plated onto 25 ml of agar for determining viable counts. The limit of viable counts was 20 CFU/ml.

Affinities for bacterial PBPs. Affinities for PBP2a of MRSA SP9099-9H (penicillinase-free, homologous resistant strain), PBP5 of *E. faecium* TL-3273 (ampicillin- and vancomycin-resistant strain), and PBPs of *P. aeruginosa* NCTC10490

TABLE 2. Binding affinities of SM-216601 and imipenem for PBP2a of *S. aureus* SP-9099-9H and PBP5 of *E. faecium* TL-3273

Carbapenem	*S. aureus* SP-9099-9H		*E. faecium* TL-3273	
	MIC	IC_{50} for PBP2a	MIC	IC_{50} for PBP5
SM-216601	2[a]	1	2	2
Imipenem	64	57	256	231

[a] Values are in micrograms per milliliter.

TABLE 3. Binding affinities of SM-216601 and meropenem for gram-negative bacterial PBPs

Strain and antibiotic	MIC (μg/ml)	IC_{50} for PBPs (μg/ml)					
		1A	1B	2	3	4	5/6
E. coli K-12 c600							
SM-216601	0.25	0.94	1.60	0.18	1.60	>3	0.54
Meropenem	0.016	0.95	1.41	0.06	0.38	0.05	>3
P. aeruginosa NCTC10490							
SM-216601	2	1.03	0.62	0.46	0.18	0.04	0.58
Meropenem	0.016	0.46	0.71	0.23	0.07	0.02	>10

and *E. coli* K-12 c600 were determined by a competition assay with PCG as previously reported (7, 23, 25, 35). Briefly, 100 μg of the bacterial membrane fraction was pretreated with drugs for 30 min at 30°C (MRSA) or 37°C (VRE) and then incubated with PCG for another 30 min, followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. For gram-negative bacteria, 300 μg of the membrane fraction was pretreated with drugs at 30°C for 10 min, followed by incubating with PCG for another 10 min. The reaction mixture was incubated at room temperature for 20 min after the addition of 2 μl of a solution containing 3 volumes of 20% sodium sarcosine and 1 volume of 180-mg/ml unlabeled PCG and centrifuged at room temperature for 30 min. The supernatant was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Binding affinity was measured by a BAS2000 Image Analyzer (Fuji Photofilms) and expressed as the 50% inhibitory concentration (IC_{50}), i.e., the concentration (in micrograms per milliliter) that inhibited radiolabeling with PCG by 50% compared with the control.

Resistance to hydrolysis by DHP-I. The resistance of SM-216601 to hydrolysis by dehydropeptidase I (DHP-I) was determined with purified mouse renal DHP-I (8) and recombinant human DHP-I, which was prepared as reported by Adachi et al. (1). The activity of DHP-I was spectrophotometrically determined by measuring the hydrolysis of glycyldehydrophenylalanine as a substrate (6). The velocity of hydrolysis of carbapenems in the presence of mouse DHP-I (4.7 mU/ml) or human DHP-I (85 mU/ml) was expressed in micromoles per minute per unit. The relative rate of hydrolysis was also calculated as a ratio against the hydrolysis rate for imipenem, which was assigned a value of 1.00.

In vivo experiments. Three-week-old male slc:ICR mice weighing 11 to 13 g were purchased from Japan SLC, Inc. (Shizuoka, Japan), and adapted to standardized environmental conditions (temperature, 23 ± 2°C; humidity, 55% ± 10%) for 1 week before the experiments. All animal procedures were performed in accordance with the institution's guidelines for the humane handling, care, and treatment of research animals. SM-216601, imipenem, and meropenem were used as a mixture with an equal dose of cilastatin, a DHP-I inhibitor, in the animal experiments unless specified otherwise. All statistical analyses described below were performed with Statistical Analysis System (SAS) version 8.01 for Windows (SAS Institute, Inc., Cary, N.C.).

(i) Experimental bacterial septicemia in mice. Mice were administered 200 mg of cyclophosphamide per kg of body weight subcutaneously 4 days before infection. Overnight cultures of *S. aureus* Smith (MSSA) and SP-12249 (MRSA) in MHA were harvested and suspended with 8% gastric mucin (Difco) in phosphate-buffered saline. A 0.2-ml aliquot of the bacterial suspension was administered intraperitoneally to each of the cyclophosphamide-pretreated mice. Two hours after infection, groups of 10 mice were injected subcutaneously with a single dose of antibiotics. For *E. coli* SP-6088 and *P. aeruginosa* IFO3451, bacterial inoculation of normal mice was conducted as described above. Consequently, groups of 10 mice were subcutaneously injected with a single dose of antibiotics 2 h after infection with *E. coli* and with three doses of antibiotics 1, 2, and 3 h after infection with *P. aeruginosa*, respectively. The 50% effective dose (ED_{50}) (in milligrams per kilogram of body weight) and 95% confidence intervals were calculated by the probit method from survival rates 7 days after infection.

(ii) Experimental *E. faecium* subcutaneous abscesses in mice. Each mouse was injected beneath the loose skin of the left groin with 0.5 ml of a bacterial diluent of *E. faecium* TL-3273 (approximately 10^8 CFU). SM-216601 and linezolid were administered subcutaneously 1 and 3 h after infection (six mice per group). The abscesses were excised 72 h after infection, at which time their formation could be confirmed visibly, and viable cell counts of the number of bacteria per abscess were made in duplicate by using standard plating procedures. Statistical analysis of the difference between drug-treated groups and a control group was performed by using Dunnett's test for multiple comparisons of significance.

(iii) Determination of plasma drug levels. A dose of 20 mg of antibiotic per kg was intravenously injected into three mice in each group. A sample of heart blood was obtained 5, 15, 30, 60, and 90 min after drug administration. The level of biologically active β-lactams in plasma was determined by the bioassay method with *Bacillus subtilis* ATCC 6633 for SM-216601 and imipenem or with *E. coli* NIHJ for meropenem and ceftazidime as the indicator organism. Standard curves were made for the antibiotics in pooled mouse serum. The disk diffusion bioassay was performed in triplicate with 50 μl of serum from β-lactam-administered mice with a standard solution. The levels of vancomycin and linezolid in plasma were determined by high-pressure liquid chromatography-UV detection. Standard curves were generated by linear regression. Samples with unknown concentrations of drugs in serum were calculated from the equation of the line. The pharmacokinetic parameters were calculated according to the moment analysis.

RESULTS

In vitro antimicrobial activity. The comparative in vitro activities of SM-216601 and other antibiotics against 396 strains of gram-positive and gram-negative clinical isolates are given in Table 1. SM-216601 was highly active against both β-lactam-susceptible and β-lactam-resistant staphylococci. Notably, the MIC at which 90% of the strains are inhibited (MIC_{90}) among 30 isolates of MRSA was 2 μg/ml and comparable to those of vancomycin (1 μg/ml) and linezolid (2 μg/ml). Twelve strains of MRSE were also susceptible to SM-216601, with MICs ranging from 0.125 to 1 μg/ml.

Penicillin-susceptible streptococci were also highly susceptible to SM-216601, the highest MIC being ≤0.016 μg/ml. The MIC_{90} of SM-216601 against a total of 14 isolates of PRSP was 0.25 μg/ml, which was twofold less than that of panipenem but comparable to that of imipenem and 16-fold and 32-fold more potent than penicillin G and flomoxef, respectively.

The MICs of SM-216601 against 12 isolates of *E. faecalis* ranged from 1 to 2 μg/ml, which were similar to those of imipenem, ampicillin, vancomycin, and linezolid and superior to those of meropenem and flomoxef. The MICs of SM-216601 against 32 isolates of *E. faecium*, including 20 vancomycin-resistant strains, ranged from 0.5 to 16 μg/ml and were lower than those of the other β-lactams tested. The MIC_{50} and MIC_{90} of SM-216601 were 4 and 8 μg/ml, which were twofold and fourfold higher than those of linezolid, respectively. The susceptibility of *E. faecium* to SM-216601 was not influenced by the vancomycin resistance phenotype (data not shown). Thus, SM-216601 proved to be the most active β-lactam antibiotic tested against those two enterococci.

SM-216601 exhibited strong activity against *E. coli*, *K. pneumoniae*, *H. influenzae*, *M. catarrhalis*, and *Proteus mirabilis*, with MIC_{90}s of less than 0.5 μg/ml, which were roughly comparable to those of imipenem or panipenem. The activity of SM-216601 against ampicillin-resistant *H. influenzae* (MIC_{90}, 0.25 μg/ml) was particularly notable. The MIC_{90}s against *Serratia marces-*

TABLE 4. Activities of SM-216601 against various β-lactamase-producing gram-negative bacteria

Enzyme and organism	β-Lactamase	MIC (μg/ml)[a]							
		SM-216601		Imipenem		Meropenem		Flomoxef	
		10^6	10^8	10^6	10^8	10^6	10^8	10^6	10^8
Cephalosporinase									
E. coli TL-3350	Group 1[b]	0.5	1	0.25	0.5	≤0.063	≤0.063	1	2
P. rettgeri TL-3363[c]	Group 1	1	2	0.5	1	≤0.063	≤0.063	≤0.063	≤0.063
E. cloacae TL-3364	Group 1	0.5	2	0.25	0.5	≤0.063	≤0.063	16	32
M. morganii TL-3365[d]	Group 1	0.5	1	2	4	≤0.063	0.125	1	8
S. marcescens TL-3367	Group 1	8	8	1	2	0.125	0.125	32	64
C. freundii TL-3368	Group 1	16	32	1	2	0.125	0.125	>128	>128
P. aeruginosa TL-3366	Group 1	8	16	2	2	0.125	0.125	>128	>128
Extended-spectrum β-lactamase									
E. coli TL-3138	Toho-1	0.5	0.5	0.125	0.25	≤0.063	≤0.063	≤0.063	≤0.063
E. coli TL-3135	Toho-2	1	1	0.25	0.25	≤0.063	≤0.063	0.125	0.125
E. coli TL-3141	MEN-1	0.5	0.5	0.25	0.5	≤0.063	≤0.063	0.125	0.125
E. coli TL-3180	SHV-12	0.25	0.25	0.25	0.5	≤0.063	≤0.063	≤0.063	≤0.063
K. pneumoniae TL-3139	MEN-1	0.25	0.5	0.25	0.5	≤0.063	≤0.063	≤0.063	0.125
K. pneumoniae TL-3149	SHV	1	2	0.5	0.5	≤0.063	≤0.063	0.125	0.125
Carbapenemase									
S. marcescens TL-3230	Group 3[b]	>128	>128	>128	>128	128	>128	>128	>128
P. aeruginosa TL-3232	Group 3	>128	>128	>128	>128	>128	>128	>128	>128
S. maltophilia TL-3370[e]	Group 3	>128	>128	>128	>128	128	>128	128	>128

[a] MICs were determined at inoculum sizes of 10^6 and 10^8 CFU/ml by the twofold agar dilution method.
[b] Bush-Jacoby-Medeiros group (5).
[c] *Providencia rettgeri.*
[d] *Morganella morganii.*
[e] *Stenotrophomonas maltophilia.*

cens, *Enterobacter cloacae*, and *Citrobacter freundii* ranged from 4 to 16 μg/ml, which were higher than those of other carbapenems but comparable to those of flomoxef. In addition, SM-216601 showed marginal activity against *P. aeruginosa*: the MIC_{90} (32 μg/ml) was two- to eightfold higher than those of other carbapenems. In terms of its effect on anaerobic bacteria, SM-216601 showed strong activity against peptostreptococci and *B. fragilis* and was similar in potency to the other carbapenems tested.

Time-kill assays. In time-kill assays, SM-216601 and quinupristin-dalfopristin caused a 2-log reduction in the CFU of *S. aureus* SP-12249 (MRSA) at the MIC and at four times the MIC (4 μg/ml) until 4 h, whereas the killing rates of vancomycin were relatively low and linezolid showed almost no bactericidal activity at any concentration tested. After 24 h of incubation, no bacterial cell was observed when SM-216601 and vancomycin were applied at four times the MIC, while the other two agents could not eradicate MRSA over the highest concentrations (Fig. 2A). For vancomycin- and ampicillin-resistant *E. faecium* TL-3273, 8 μg/ml of SM-216601 caused a 4-log reduction after 24 h; its killing activity was the greatest among the compounds tested (Fig. 2B). SM-216601 and the other carbapenems tested were highly bactericidal against *S. pneumoniae* 181. A four-log reduction by those carbapenems at concentrations at the MIC and 1/4× the MIC was achieved at 6 h. The bactericidal activity of vancomycin was slightly weaker than that of any carbapenem. Ampicillin exhibited a potent killing effect similar to that of the carbapenems, but its effective concentration was much higher (Fig. 2C). SM-216601 caused a time-dependent reduction in viable cells of *H. influenzae* 231,

TABLE 5. Activity of SM-216601 against *S. aureus* systemic infection in immunosuppressed mice[a]

Strain	No. of CFU/mouse (multiple of LD_{50})[b]	Antimicrobial agent	MIC (μg/ml)	ED_{50}, mg/kg (95% confidence limits)
Smith (MSSA)	1.12×10^5 (60.2)	SM-216601	0.016	0.09 (0.05–0.14)
		Vancomycin	1	2.40 (1.11–4.57)
		Linezolid	2	17.16 (ND)[c]
		Imipenem	0.016	0.07 (0.03–0.13)
SP-12249 (MRSA)	1.13×10^6 (20.3)	SM-216601	1	2.89 (1.52–5.35)
		Vancomycin	1	0.89 (0.58–1.54)
		Linezolid	1	4.15 (2.40–7.78)
		Imipenem	32	14.93 (8.10–26.28)

[a] Ten cyclophosphamide-pretreated mice in each group received a single subcutaneous dose of the agent 2 h after a bacterial challenge.
[b] LD_{50}, 50% lethal dose.
[c] ND, not determined.

TABLE 6. Activities of SM-216601 against *E. coli* and *P. aeruginosa* systemic infections in mice[a]

Strain	No. of CFU/mouse (multiple of LD_{50})[b]	Antimicrobial agent	MIC (μg/ml)	ED_{50}, mg/kg (95% confidence limits)
E. coli SP-6088	7.56×10^5 (31.6)	SM-216601	0.125	2.64 (1.58–4.55)
		Meropenem	0.016	1.57 (0.89–2.85)
		Ceftazidime	0.063	4.66 (2.67–9.09)
P. aeruginosa IFO3451	5.94×10^6 (2.5)	SM-216601	8	20.95 (13.1–36.0)
		Meropenem	0.5	0.89 (0.58–1.54)
		Ceftazidime	1	>60 (ND)[c]

[a] Ten mice in each group received a single subcutaneous dose of the agent 2 h after an *E. coli* challenge or three doses of the agent 1, 2, and 3 h after a *P. aeruginosa* challenge, respectively.
[b] LD_{50}, 50% lethal dose.
[c] ND, not determined.

which is a β-lactamase-negative, ampicillin-resistant strain, at the MIC and four times the MIC; its killing kinetics was similar to those of ampicillin and meropenem, but the MIC of SM-216601 was the lowest of the three β-lactams. Meanwhile, cefotaxime and imipenem were less bactericidal than SM-216601. Notably, imipenem did not show sufficient killing activity, even at four times the MIC (Fig. 3A). Although the MIC of SM-216601 against *P. aeruginosa* IFO3451 was two- to eightfold higher than the MICs of other carbapenems, SM-216601 achieved a time-dependent decrease in the number of viable cells at concentrations above the MIC. The killing activity of SM-216601 was similar to those of existing carbapenems (Fig. 3B).

Affinity for bacterial PBPs. The IC_{50}s of SM-216601 and imipenem for PBP2a of MRSA strain SP-9099-9H and for PBP5 of vancomycin- and ampicillin-resistant *E. faecium* TL-3273 were determined by using a competition assay with PCG (Table 2). The MIC of SM-216601 for both strains was 2 μg/ml and correlated well with the corresponding IC_{50}s, 1 μg/ml against MRSA and 2 μg/ml against *E. faecium*. A comparison of the IC_{50}s and corresponding MICs of SM-216601 and imipenem revealed that the improvement in the anti-MRSA and anti-*E. faecium* activities of SM-216601 could be ascribed to the activity against those low-affinity PBPs.

The binding affinities of SM-216601 and meropenem for the PBPs of *E. coli* K-12 c600 and *P. aeruginosa* NCTC10490 are shown in Table 3. As for *E. coli*, SM-216601 exhibited potent binding affinities for the PBPs, except for PBP4; the IC_{50}s of PBP2 (0.18 μg/ml) and PBP5/6 (0.54 μg/ml) showed a good correlation with the MIC (0.25 μg/ml). In accordance with a previous study (24), meropenem strongly bound PBP2 and PBP4 but not PBP5/6 of *E. coli*. The IC_{50}s of SM-216601 for PBP1, -2, -3, and -4 of *P. aeruginosa* were two- to threefold higher than those of meropenem, but SM-216601 exhibited a higher affinity for PBP 5/6 than did meropenem. Thus, an interesting difference in affinity for gram-negative bacterial PBPs was observed between the two carbapenems. However, the affinities of SM-216601 and meropenem for the PBPs of *P. aeruginosa* could not fully explain the differences among the MICs of the carbapenems tested.

TABLE 7. Activity of SM-216601 against experimental *E. faecium* TL-3273 subcutaneous abscesses in mice[a]

Antimicrobial agent	Dose (mg/kg)	Mean log CFU count at site of infection ± SD	Decrease in log CFU count vs control	*P* value[b]
None (control)		7.46 ± 0.21		
SM-216601	7.5	6.43 ± 0.22	−1.03	<0.0001
	20	5.18 ± 0.16	−2.28	<0.0001
	40	5.08 ± 0.15	−2.38	<0.0001
Linezolid	40	6.72 ± 0.15	−0.74	<0.05

[a] Six mice in each group received two subcutaneous doses of SM-216601 (MIC, 2 μg/ml) or linezolid (MIC, 2 μg/ml) 1 and 3 h after the subcutaneous inoculation of *E. faecium* TL-3272. Viable-cell counts of the bacteria per abscess were made 3 days after infection.
[b] Dunnett's test for multiple comparisons of significance versus control.

Antimicrobial activity against β-lactamase-producing bacteria. As shown in Table 4, SM-216601 maintained potent activity against *E. coli*, *Providencia rettgeri*, *E. cloacae*, and *Morganella morganii* producing cephalosporinase (group 1 β-lactamase). The MICs of SM-216601 were higher than those of imipenem for other cephalosporinase-producing strains but still lower than those of flomoxef, even with a large inoculum of bacteria (10^8 CFU/ml). In addition, SM-216601 exhibited good activity against *E. coli* and *K. pneumoniae* producing extended-spectrum β-lactamases. However, there was no activity against carbapenemase (group 3 β-lactamase) producers, consisted with the results obtained with imipenem and meropenem.

Resistance to hydrolysis by DHP-I. In advance of animal studies, the resistance of SM-216601 to enzymatic hydrolysis was tested with renal DHP-I from mice and recombinant human DHP-I. The rate of SM-216601 hydrolysis by mouse DHP-I was 0.803 μmol/min/U, and that by human DHP-I was 0.029 μmol/min/U. These DHP-I susceptibilities were similar to those of meropenem (0.820 μmol/min/U by mouse DHP-I and 0.027 μmol/min/U by human DHP-I) but significantly different from those of imipenem (0.499 and 0.082 μmol/min/U, respectively). Since, in contrast to human DHP-I, SM-216601 was more susceptible to mouse DHP-I than imipenem was, we coadministered equal amounts of cilastatin with SM-216601 and other carbapenems for the following in vivo studies to avoid a species-specific effect by DHP-I on the metabolism of carbapenems in mice.

In vivo efficacy against gram-positive and gram-negative bacteria. The therapeutic efficacy of SM-216601 (with cilastatin) against systemic infections with MSSA and MRSA in immunosuppressed mice was determined (Table 5). The MICs of SM-216601, vancomycin, linezolid, and imipenem against *S.*

TABLE 8. Pharmacokinetic parameters of SM-216601 in mice[a]

Antimicrobial agent	$C_{5\ min}$ (μg/ml)	$t_{1/2}$ (min)	CL[b] (ml/min/kg)	V_{ss}[c] (μg/kg)	$AUC_{0-\infty}$[d] (μg · min/ml)
Expt 1					
SM-216601	74.6	17.8	9.7	0.26	2,054
Vancomycin	37.8	16.5	19.4	0.48	1,034
Linezolid	26.0	54.9	11.8	0.94	1,692
Imipenem	53.8	7.7	26.2	0.34	762
Expt 2					
SM-216601	66.2	20.8	9.9	0.31	2,026
Meropenem	40.0	10.1	29.0	0.44	691
Ceftazidime	27.8	11.6	34.1	0.61	587

[a] Three mice received a single intravenous dose (20 mg/kg) of each agent.
[b] CL, clearance.
[c] V_{ss}, volume of distribution at steady state.
[d] $AUC_{0-\infty}$, AUC from 0 min to infinity.

aureus strain Smith were 0.016, 1, 2, and 0.016 μg/ml, respectively. The ED_{50} of SM-216601 against infection with the strain was 0.09 mg/kg, which was comparable to that of imipenem and much lower than the ED_{50} of vancomycin or linezolid. Meanwhile, SM-216601 showed in vitro activity against the MRSA SP-12249 strain comparable to those of vancomycin and linezolid, with an MIC of 1 μg/ml. The ED_{50} of SM-216601 against MRSA infection was 2.89 mg/kg, equivalent to the values for vancomycin and linezolid. In this model, imipenem showed little protective activity (ED_{50} = 14.93 mg/kg), which seemed to be consistent with its low level of in vitro activity. These results suggested that the in vivo efficacy of SM-216601 against these MSSA and MRSA strains was consistent with the corresponding level of in vitro activity. SM-216601 also had potent efficacy against gram-negative bacterial infections in mice (Table 6). The ED_{50}s of SM-216601 against systemic infection with *E. coli* SP-6088 and *P. aeruginosa* IFO3451 were between those of meropenem and ceftazidime, regardless of the MICs of SM-216601, which were higher than those of meropenem or ceftazidime. Thus, the difference in in vivo efficacy between SM-216601 and ceftazidime was not reflected in their in vitro efficacies, even considering the favorable pharmacokinetics of SM-216601 in mice indicated by a comparison of the concentration of the drug 5 min after administration ($C_{5\ min}$) and its elimination half-life ($t_{1/2}$) to those of ceftazidime, which might be expected to result in a prolonged time above the MIC of SM-216601 (see Table 8).

We next determined the efficacy of SM-216601 against *E. faecium* by using a subcutaneous abscess mouse model (Table 7). The strain of *E. faecium* used in this model, TL-3273, exhibited reduced susceptibility to ampicillin and vancomycin (MICs, 128 μg/ml and 256 μg/ml, respectively) but was susceptible to SM-216601 and linezolid (both MICs, 2 μg/ml). This strain formed visible abscesses in mice at 3 days after subcutaneous inoculation of approximately 10^8 CFU of bacteria, and the number of organisms in abscesses of untreated mice ranged from 10^7 to 10^8 CFU in multiple experiments. SM-216601 dose dependently reduced bacterial numbers in abscesses following two subcutaneous injections of 7.5, 20, and 40 mg/kg/dose and caused a more-than-2-log reduction at the highest dose. Linezolid also caused a 0.5-log reduction of bacterial numbers at 40 mg/kg/dose. Its activity at 40 mg/kg/dose was weaker than even that of SM-216601 at 7.5 mg/kg/dose.

Pharmacokinetics in mice. The pharmacokinetics of SM-216601 administered intravenously at a dose of 20 mg/kg are compared with those of imipenem, vancomycin, and linezolid (experiment 1) and those of meropenem and ceftazidime (experiment 2) in Table 8. The $C_{5\ min}$s of SM-216601, imipenem, vancomycin, and linezolid were 74.6, 53.8, 37.8, and 26.0 μg/ml, respectively. The area under the plasma concentration-time curve (AUC) for SM-216601 was 2,054 μg min/ml, the value being 1.2- to 2.7-fold higher than the values of the reference agents tested. The $t_{1/2}$ of SM-216601 was 17.8 min, whereas those of imipenem, vancomycin, and linezolid were 7.7, 16.5, and 54.9 min, respectively. SM-216601 exhibited a notably higher $C_{5\ min}$ and AUC than those of meropenem and ceftazidime. In addition, the $t_{1/2}$ of SM-216601 (20.8 min) was approximately twofold longer than those of meropenem (10.1 min) and ceftazidime (11.6 min).

DISCUSSION

A new parenteral carbapenem, SM-216601, exhibited improved activity against methicillin-resistant staphylococci and *E. faecium*, distinguishing it from existing carbapenems. The notably high affinities of SM-216601 for PBP2a of MRSA and PBP5 of ampicillin-resistant *E. faecium* suggest the importance of the C-2 side chain containing a lipophilic thiazole moiety for binding to these low-affinity PBPs and consequently for sufficient antibacterial activities.

SM-216601 was highly active against *E. coli*, *K. pneumoniae*, *M. catarrhalis*, and ampicillin-resistant *H. influenzae*, which are frequently isolated as causative bacteria from patients with a variety of nosocomial infections, especially respiratory infections. In addition, SM-216601 was less active against *E. cloacae*, *C. freundii*, *S. marcescens*, and *P. aeruginosa* than were other carbapenems, but the activity was still comparable to or higher than that of the cephalosporin flomoxef.

We previously reported that the anti-gram-negative bacterial activities of a series of 2-thiazolcarbapenem derivatives, including SM-216601, were correlated with their physicochemical properties, which were well known to affect the outer membrane permeability of the carbapenems (33). For instance, increasing the lipophilicity of the C-2 side chain tended to reduce the antibacterial activities against *E. cloacae*, *C. freundii*, *S. marcescens*, and *P. aeruginosa*, which were bacterial spe-

cies possessing low outer membrane permeability. Although a substantial difference in PBP affinity was observed between SM-216601 and meropenem in this study (Table 3), it is not likely that this explains the difference in the MICs among carbapenems tested against *E. coli* and *P. aeruginosa*. Thus, these observations suggest that the outer membrane permeability of SM-216601 affects its anti-gram-negative bacterial activity.

It is well known that carbapenems mainly pass through the outer membrane of *P. aeruginosa* via a specific porin channel, the outer membrane D_2 protein (OprD) (29, 31), and that the uptake of carbapenems by OprD is interfered with by basic amino acids in the culture medium (30), which thus results in a reduction in the antipseudomonal activity (19). In this context, we compared the MICs of SM-216601 against the *P. aeruginosa* PAO1 strain, which has been extensively studied in previous reports (9), between MHA and nutrient agar (NA). As a result, the MIC of SM-216601 in NHA was 16 μg/ml while the MIC was 1 μg/ml (16-fold decrease) in NA, whose basic amino acid content was lower than that of MHA. In addition, the degree of MIC change of SM-216601 in NA was more pronounced than those of imipenem (twofold decrease), meropenem (eightfold decrease), and panipenem (fourfold decrease). These unpublished observations may suggest the involvement of OprD in the uptake of SM-216601 by *P. aeruginosa*. Furthermore, meropenem but not imipenem has been reported to be a substrate of certain efflux systems in the outer membrane of *P. aeruginosa*, and overexpression of efflux pumps results in reduced susceptibility to meropenem (17). Due to the structural similarities of SM-216601 to meropenem, such as the presence of the 1β-methyl group and a relatively weak basic C-2 side chain, the possible involvement of an efflux system(s) in its antipseudomonal activity cannot be excluded.

The in vivo efficacies of SM-216601 in a murine septicemia model of MRSA and a subcutaneous abscess model of *E. faecium* seemed to reflect its potent in vitro activities against both bacterial strains tested. The efficacies of SM-216601 were stronger than those of linezolid, although these agents had the same MICs. In contrast to the bacteriostatic activities of linezolid against those strains, the bactericidal activity of SM-216601 might contribute to such high in vivo efficacies. The ED_{50}s of SM-216601 against murine systemic infections with *E. coli* and *P. aeruginosa* were higher than those of meropenem, and the difference between the two carbapenems may be consistent with their in vitro activities. On the other hand, SM-216601 was more potent than ceftazidime in those models although its MICs were higher than those of ceftazidime. As shown in Table 8, the better pharmacokinetics of SM-216601 than that of ceftazidime in mice could be an explanation. However, the significant difference between the in vivo efficacies of meropenem and ceftazidime, their MICs and pharmacokinetic parameters in mice being similar, suggests that another factor(s) superior to cephalosporins, such as the time above the MIC necessary to exhibit antibacterial effects in mice, might have a positive impact on the efficacies of both SM-216601 and meropenem.

Since the 1990s, nosocomial infections with multiresistant gram-positive bacteria have been recognized as a serious problem because the treatment against such pathogens has been limited (4). In addition, since multiresistant gram-positive bacteria often cause polymicrobial infections with gram-negative bacteria, superinfection is a concern for the long-term treatment of narrow-spectrum antibacterials such as glycopeptides or an oxazolidinone. In this context, combination therapy with vancomycin or linezolid and broad-spectrum agents such as expanded-spectrum cephalosporins have been used for empirical therapy in severe cases of nosocomial infections in which the involvement of multiresistant gram-positive pathogens is suspected. However, the risk of adverse reactions and undesired progression of drug resistance due to the overuse of narrow-spectrum or bacteriostatic agents is considered a major drawback of this kind of treatment (2b). Thus, monotherapy with a highly tolerated agent effective against both multiresistant gram-positive bacteria and major gram-negative bacteria is highly desirable. Although a number of new cephalosporin and carbapenem compounds targeting MRSA have been disclosed (11, 27), the development of a broad-spectrum β-lactam still appears challenging. An increase in the anti-MRSA activity of β-lactam compounds often results in a reduction in the level of activity against gram-negative bacteria, with few exceptions (2a, 10, 12–15, 20, 32, 34). In addition, improving the activity against *E. faecium* is also difficult and only a few β-lactams have been reported to be effective against *E. faecium* (18, 25, 34).

SM-216601 showed an antibacterial activity which extends to MRSA and VRE, in comparison with existing carbapenems. Moreover, this carbapenem exhibited better pharmacokinetics than imipenem and meropenem in mice. Similar pharmacokinetics were observed in rats, dogs, and cynomolgus monkeys (data not shown), suggesting a long-acting pharmacokinetic profile of SM-216601 in humans. In conclusion, SM-216601 should be a promising candidate as a broad-spectrum β-lactam antibiotic for the treatment of nosocomial infections by a number of gram-positive and gram-negative bacteria, especially multiresistant pathogens, including MRSA and VRE.

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