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Antimicrobial Activity of SM-17466, a Novel Carbapenem Antibiotic with Potent Activity against Methicillin-Resistant *Staphylococcus aureus*

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The in vitro and in vivo antibacterial activities of SM-17466, a new 1β-methyl carbapenem, were evaluated against a wide range of clinical bacterial isolates and compared with the activities of meropenem, imipenem, vancomycin, and arbekacin. SM-17466 had a broad spectrum of action against gram-positive bacteria, showing especially potent activity against methicillin-resistant staphylococci. The MICs of SM-17466, meropenem, imipenem, vancomycin, and arbekacin at which 90% of clinical isolates of methicillin-resistant *Staphylococcus aureus* were inhibited were 3.13, 50, 100, 1.56, and 3.13 μg/ml, respectively. This activity of SM-17466 was almost equivalent to those of the antibiotics used for the treatment of infections caused by this organism. SM-17466 also showed bactericidal activity against methicillin-resistant *S. aureus*. In contrast, SM-17466 was less active against gram-negative bacteria, especially against *Pseudomonas aeruginosa*, compared with the other carbapenems; however, of the carbapenems, SM-17466 exhibited the highest activity against *Haemophilus influenzae* and *Bacteroides fragilis*. SM-17466, at a 50% inhibitory concentration of less than 1 μg/ml, bound to penicillin-binding proteins 1 to 4 in methicillin-susceptible *S. aureus* and also had good binding to penicillin-binding protein 2′ in a methicillin-resistant strain (50% inhibitory concentration, 5.9 μg/ml). This high affinity, which was 10 and 20 times greater than those for meropenem and imipenem, respectively, was reflected in the potent activity of SM-17466 against methicillin-resistant *S. aureus*. SM-17466 demonstrated excellent in vivo efficacy against methicillin-susceptible and -resistant *S. aureus* strains in a mouse peritoneal infection model: the efficacy of SM-17466 against methicillin-resistant strains was equal to or one-third that of vancomycin. This activity was comparable to the in vitro activity of SM-17466. The subcutaneous injection of SM-17466 in mice revealed that the half-life of SM-17466 in serum was about 18 min, intermediate between those of vancomycin and arbekacin and 1.5-fold that of imipenem-cilastatin. SM-17466 was resistant to hydrolysis by swine renal dehydropeptidase I, to an extent comparable to the resistance shown by meropenem.

Because of their wide spectrum and high activity against many pathogens, including *Pseudomonas aeruginosa*, carbapenems have opened new fields for the use of β-lactam antibiotics. Imipenem was the first carbapenem antibiotic used clinically. Subsequently, many carbapenems, such as panipenem (RS-533) (5, 14), meropenem (3, 19), biapenem (L-627) (2, 22, 24), and BO-2727 (13) have been developed and used for the treatment of severe infections or in clinical trials. The emergence of, and now the epidemic nature, of methicillin-resistant *Staphylococcus aureus* is a serious problem in antibacterial chemotherapy (23). However, the carbapenems noted above do not show high activity against this highly resistant organism. Methicillin-resistant *S. aureus* is a major pathogen in patients who have nosocomial infections (15). Although vancomycin has proven to be useful for treating staphylococcal infections, therapy with this drug is relatively limited because of its adverse effects, poor oral absorption, and relatively low cost-effectiveness.

In the process of research for new carbapenem compounds with activity against methicillin-resistant *S. aureus*, we synthesized a new 1β-methyl carbapenem, SM-17466, (4*R*,5*S*,6*S*)-3-{[4-(1-aminocarbonyl-methyl-pyridino-4-yl)thiazol-2-yl]thio}-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3,2,0]hept-2-ene-2-carboxylate (Fig. 1). The introduction of a pyridinothiazole group into the 2 position led to high activity against methicillin-resistant *S. aureus*, and the 1β-methyl and quaternized groups achieved stability against renal dehydropeptidase I (DHP-I).

In this report, we describe the in vitro antibacterial activities of SM-17466 against clinical isolates and the in vivo activity of SM-17466 against methicillin-resistant *S. aureus* strains, compared with the activities of meropenem, imipenem, vancomycin, and arbekacin (10). The latter two agents are used for the treatment of infections caused by methicillin-resistant *S. aureus*. We also investigated the stability of SM-17466 in the presence of DHP-I.

MATERIALS AND METHODS

Antibiotics. SM-17466, meropenem, imipenem, and panipenem were synthesized in the laboratories of Sumitomo Pharmaceuticals Research Center, Osaka, Japan. Glycyldehydrophenylalanine was also prepared in these laboratories. Vancomycin and arbekacin were obtained from Shionogi & Co., Ltd. (Osaka, Japan), and Meiji Seika Kaisha Ltd. (Tokyo, Japan), respectively. Other antimicrobial agents were obtained from other commercial sources. Antimicrobial agents were freshly prepared on the day of use. [^{14}C]benzylpenicillin potassium salt (59 mCi/mmol) was purchased from Amersham International plc. (Bucks, United Kingdom).

Organisms. The clinical bacterial isolates used in this study were collected from several hospitals in Japan over the past few years. *S. aureus* strains used for the in vivo study were selected from the stock cultures held in this laboratory. All organisms were maintained in glycerol broth at −80°C.

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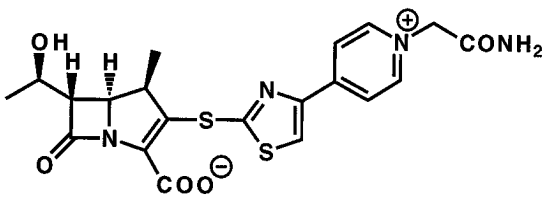

FIG. 1. Chemical structure of SM-17466.

Susceptibility testing. MICs were determined by the twofold serial agar dilution method, with Mueller-Hinton medium (Difco Laboratories, Detroit, Mich.), supplemented with 5% defibrinated horse blood for streptococci and with 5% Fildes enrichment (Difco) for *Haemophilus influenzae*. For *Bacteroides fragilis*, GAM agar (Nissui Pharmaceutical, Tokyo, Japan) was used.

An overnight broth culture or bacterial suspension was diluted with the corresponding broth or with phosphate-buffered saline supplemented with 0.01% gelatin to give a final concentration of approximately 10^7 CFU/ml. A portion (about 5 μl) of the dilution was plated onto a drug-containing agar surface with an inoculum apparatus (Microplanter; Sakuma Seisakusho, Tokyo, Japan). The final inoculum size was approximately 10^5 CFU per spot. The plates were incubated at 37°C for 20 h, except for methicillin-resistant staphylococci (incubated at 35°C for 24 h in Mueller-Hinton medium supplemented with 4% NaCl), *H. influenzae* (cultured in the presence of 5% CO_2), and *B. fragilis* (incubated at 37°C for 24 h in GasPack jars [BBL Microbiology System, Cockeysville, Md.]). MIC was defined as the lowest antibiotic concentration that completely prevented visible growth.

The degree of homogeneity of phenotypic resistance in *S. aureus* was assessed by disk diffusion tests and population analysis (1).

Bactericidal activity. The bactericidal activities of the drugs against homogeneous resistant *S. aureus* were assessed by a broth dilution technique. An overnight culture of the test organism was diluted with fresh Mueller-Hinton broth (Difco) to about 10^6 CFU/ml, and 0.9 ml of the dilution was added to 0.1 ml of the drug solution in a clear tube; the tubes were then incubated at 37°C for 24 h and inspected for turbidity. The number of inoculated cells was confirmed to be 5×10^5 to 1×10^6 CFU/ml. After the determination of MICs, 0.01-ml samples of growth-negative cultures were removed to antibiotic-free plates, which were then incubated for 48 h at 35°C for colony formation. MBC was defined as the lowest drug concentration capable of reducing the initial inoculum to <99.9%; the initial inoculum and reduction in numbers were assessed by counting viable cells. We confirmed that the drug carryover did not affect colony formation.

Affinity for PBPs. The affinities of penicillin-binding proteins (PBPs) 1 to 4 and PBP 2′ for SM-17466 were assessed separately with methicillin-susceptible *S. aureus* FDA209P and with methicillin-resistant MS9406-6H (a penicillinase-free, homogenous resistant strain), respectively. *S. aureus* membrane fractions were prepared as previously described (18). The relative affinities of PBPs 1 to 4 for each compound were determined by a competition assay with [^{14}C]benzylpenicillin, as described previously (16, 18). The binding of carbapenem to PBP 2′ was independently determined by the method of Sumita et al. (17). Membrane fractions were pretreated with various concentrations of antibiotics for 30 min at 30°C, the final concentration of 300 μg of radioactive benzylpenicillin (diluted fivefold with nonradioactive benzylpenicillin) per ml was then added, and the fractions were incubated for another 30 min at 30°C. This procedure allowed the specific detection of PBP 2′, despite the similar molecular weights of PBPs 2′, 2, and 3.

In vivo activity. The in vivo antibacterial activities of the drugs were determined against *S. aureus* peritoneal infections in mice. Ten 4-week-old male slc:ICR mice (weight, 24 to 26 g; Japan SLC Inc., Shizuoka, Japan) were used for each dose level of the drug *S. aureus*, grown to late exponential phase in tryptic soy broth (Difco), was harvested, and cells were washed with phosphate-buffered saline. Subsequently, cells of the methicillin-susceptible *S. aureus* Smith were resuspended in 5% gastric mucin (Difco), and *S. aureus* cells of the homogeneous methicillin-resistant strains were resuspended in 8% gastric mucin. A 0.2-ml portion of the bacterial suspension, corresponding to a dose 1 to 10 times higher than the minimal lethal dose, was inoculated intraperitoneally in each mouse. Under these conditions, all untreated mice died within 3 days. Two hours after infection, mice received a subcutaneous injection of a single dose of an antibiotic. The number of organisms in the intraperitoneal cavity at the point that the antibiotic was administered was approximately 10 to 30% of the number inoculated. The number of mice surviving at each dose was counted 7 days after infection. The 50% effective dose was calculated by the Probit method (12).

Pharmacokinetic study. Groups of mice were dosed subcutaneously with SM-17466, vancomycin, and arbekacin, all at 25 mg/kg of body weight. Three mice in a group were used at each time point. Blood was obtained by retro-orbital bleeding into heparinized capillary pipettes. Samples were stored in an ice water bath until assay (about 2 h). Antibiotic concentrations in serum were determined by a disk diffusion bioassay using *Bacillus subtilis* ATCC 6633. Standard curves were made for the antibiotic in pooled mouse serum. The disk diffusion bioassay was performed in triplicate with 50 μl of serum from antibiotic-treated mice or with standard solution. Standard curves were generated by linear regression. Samples with unknown concentrations of drugs in serum were calculated from the equation of the line. The lower limits of detection for the antibiotics (in micrograms per milliliter) were as follows: SM-17466, ≤0.05; vancomycin, 1.56; arbekacin, 0.2. Pharmacokinetic parameters were calculated according to the open one-compartment model.

Stability against renal DHP-I. The stability of SM-17466 against renal DHP-I was determined with purified swine renal DHP-I, as reported previously (4). The activity of DHP-I was spectrophotometrically determined by measuring the hydrolysis of glycyldehydrophenylalanine as a substrate. The relative rate of hydrolysis was expressed as a ratio against the rate for meropenem, which was assigned a value of 1.0.

RESULTS

Antimicrobial activity against clinical isolates. The activities of SM-17466, meropenem, imipenem, vancomycin, arbekacin, and reference antibiotics against recent clinical isolates of 19 species of bacteria are given in Table 1. Of the drugs tested, SM-17466 showed good activity against gram-positive cocci such as *Staphylococcus*, *Streptococcus*, and *Enterococcus* spp. SM-17466 and imipenem were the most active agents against methicillin-susceptible staphylococci. SM-17466 also exhibited potent activity against methicillin-resistant staphylococci, especially against homogeneous resistant strains, showing MICs at which 90% of isolates were inhibited of 3.13 μg/ml for *S. aureus* and 6.25 μg/ml for *Staphylococcus epidermidis*. This activity was 8 and 32 times greater than those of meropenem and imipenem, respectively, and was slightly less than those of vancomycin and arbekacin, which are used for the treatment of infections caused by methicillin-resistant staphylococci. Of the antibiotics tested, SM-17466 had the highest activity against streptococci, similar to that of imipenem. SM-17466 inhibited the growth of penicillin-resistant *Streptococcus pneumoniae* at 0.1 μg/ml, whereas imipenem did not. These two carbapenems were also active against *Enterococcus faecalis*. Of the carbapenems tested, SM-17466 had the highest activity against *Enterococcus faecium*. However, its activity was less than that of vancomycin. In general, SM-17466 showed activity equivalent to or higher than that of imipenem against gram-positive organisms.

In contrast, SM-17466 had less activity against gram-negative bacteria than meropenem and imipenem. The activities of SM-17466 against *Escherichia coli* and *Klebsiella pneumoniae* were comparable to those of imipenem and arbekacin, inhibiting at a concentration of less than 1 μg/ml. Against *Proteus* spp. and *Morganella morganii*, SM-17466 also showed the same activity as imipenem. However, the activities of SM-17466 against organisms of these four genera were less than one-eighth of those of meropenem. The activity of SM-17466 was quite inferior to those of meropenem and imipenem against *Citrobacter freundii*, *Enterobacter cloacae*, *Serratia marcescens*, and *P. aeruginosa*, showing MICs at which 90% of the isolates were inhibited of 12.5, 12.5, 50, and 50 μg/ml, respectively. Against *H. influenzae* and *B. fragilis*, however, SM-17466 was the most active of the tested antibiotics, inhibiting at 0.025 and 0.20 μg/ml, respectively.

Bactericidal activity. The results, expressed as a multiple of the MIC, showed that the MBC of SM-17466 was about twice the MIC for methicillin-resistant *S. aureus* (Table 2). Of the drugs tested, vancomycin showed the most potent bactericidal activity. Arbekacin displayed lower bactericidal activity against these methicillin-resistant isolates. In the comparison of bactericidal activity relative to MIC, SM-17466 and meropenem had comparable results. However, the bactericidal activity of SM-17466 was 16 times higher than that of meropenem. In the population analysis, it was revealed that no homogeneous re-

TABLE 1. Antibacterial activities of SM-17466 and reference compounds against clinical isolates

Organism (no. of strains)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Staphylococcus aureus				
Methicillin susceptible (23)	SM-17466	0.013–0.025	0.013	0.025
	Meropenem	0.05–0.20	0.10	0.20
	Imipenem	0.013–0.025	0.013	0.025
	Vancomycin	0.78–1.56	0.78	1.56
	Arbekacin	0.10–1.56	0.20	0.39
	Methicillin	1.56	1.56	1.56
Methicillin resistant				
Heterogeneous (6)	SM-17466	0.39–1.56		
	Meropenem	3.13–12.5		
	Imipenem	1.56–12.5		
	Vancomycin	1.56		
	Arbekacin	0.20–0.78		
	Methicillin	>100		
Homogeneous (47)	SM-17466	1.56–6.25	3.13	3.13
	Meropenem	12.5–100	50	50
	Imipenem	25–>100	100	100
	Vancomycin	0.78–3.13	1.56	1.56
	Arbekacin	0.20–6.25	0.39	3.13
	Methicillin	>100	>100	>100
Staphylococcus epidermidis				
Methicillin susceptible (18)	SM-17466	0.013–0.20	0.10	0.10
	Meropenem	0.05–1.56	0.78	1.56
	Imipenem	0.013–0.39	0.10	0.20
	Vancomycin	1.56	1.56	1.56
	Arbekacin	0.05–1.56	0.20	0.78
	Methicillin	0.39–6.25	3.13	6.25
Methicillin resistant				
Heterogeneous (9)	SM-17466	0.39–1.56		
	Meropenem	6.25–25		
	Imipenem	3.13–12.5		
	Vancomycin	1.56		
	Arbekacin	0.10–1.56		
	Methicillin	>100		
Homogeneous (11)	SM-17466	0.78–6.25	3.13	6.25
	Meropenem	12.5–100	50	100
	Imipenem	25–>100	50	100
	Vancomycin	0.78–3.13	1.56	1.56
	Arbekacin	0.20–1.56	0.78	1.56
	Methicillin	>100	>100	>100
Streptococcus pyogenes (8)	SM-17466	≤0.006–0.013		
	Meropenem	≤0.006–0.013		
	Imipenem	≤0.006–0.013		
	Vancomycin	0.20–0.39		
	Arbekacin	12.5–50		
	Ampicillin	0.013–0.025		
Streptococcus pneumoniae				
Penicillin susceptible (11)	SM-17466	≤0.006–0.013	≤0.006	≤0.006
	Meropenem	0.013–0.05	0.013	0.025
	Imipenem	≤0.006–0.013	≤0.006	≤0.006
	Vancomycin	0.20–0.39	0.20	0.39
	Arbekacin	12.5–50	25	50
	Ampicillin	0.013–0.10	0.025	0.05
	Benzylpenicillin	≤0.006–0.05	0.013	0.05
Penicillin-resistant (11)	SM-17466	0.05–0.10	0.05	0.10
	Meropenem	0.20–0.39	0.20	0.39
	Imipenem	0.10–0.20	0.10	0.20
	Vancomycin	0.10–0.39	0.20	0.20
	Arbekacin	12.5–25	25	25
	Ampicillin	0.78–3.13	0.78	1.56
	Benzylpenicillin	1.56–3.13	1.56	3.13

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TABLE 1—*Continued*

Organism (no. of strains)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Enterococcus faecalis (14)	SM-17466	0.10–3.13	1.56	3.13
	Meropenem	3.13–25	6.25	25
	Imipenem	0.39–3.13	1.56	1.56
	Vancomycin	0.78–3.13	1.56	3.13
	Arbekacin	3.13–100	50	100
Enterococcus faecium (11)	SM-17466	3.13–12.5	6.25	12.5
	Meropenem	>100	>100	>100
	Imipenem	100–>100	>100	>100
	Vancomycin	0.78	0.78	0.78
	Arbekacin	6.25–12.5	6.25	12.5
Escherichia coli (27)	SM-17466	0.05–0.78	0.10	0.39
	Meropenem	≤0.006–0.025	0.013	0.013
	Imipenem	0.05–0.39	0.10	0.20
	Arbekacin	0.39–1.56	0.78	0.78
Klebsiella pneumoniae (15)	SM-17466	0.05–0.78	0.20	0.39
	Meropenem	0.013–0.20	0.025	0.05
	Imipenem	0.10–0.78	0.10	0.20
	Arbekacin	0.20–0.78	0.39	0.78
Proteus spp. (15)[b]	SM-17466	0.39–3.13	0.39	3.13
	Meropenem	0.05–0.10	0.10	0.10
	Imipenem	0.39–6.25	1.56	6.25
	Arbekacin	0.39–6.25	1.56	3.13
Morganella morganii (15)	SM-17466	0.39–12.5	1.56	12.5
	Meropenem	0.05–0.20	0.10	0.20
	Imipenem	3.13–6.25	3.13	6.25
	Arbekacin	0.20–6.25	1.56	3.13
Citrobacter freundii (14)	SM-17466	1.56–25	3.13	12.5
	Meropenem	0.013–0.39	0.025	0.20
	Imipenem	0.10–0.78	0.20	0.39
	Arbekacin	0.39–100	0.78	12.5
Enterobacter cloacae (15)	SM-17466	0.78–12.5	6.25	12.5
	Meropenem	0.013–0.10	0.05	0.10
	Imipenem	0.05–0.20	0.20	0.20
	Arbekacin	0.39–25	0.78	12.5
Serratia marcescens (15)	SM-17466	3.13–100	25	50
	Meropenem	0.025–12.5	1.56	6.25
	Imipenem	0.39–1.56	1.56	1.56
	Arbekacin	0.78–50	6.25	50
Pseudomonas aeruginosa (14)	SM-17466	3.13–50	12.5	50
	Meropenem	0.10–1.56	0.20	0.78
	Imipenem	0.20–1.56	0.78	1.56
	Arbekacin	0.10–6.25	3.13	6.25
Haemophilus influenzae (12)	SM-17466	0.013–0.025	0.013	0.025
	Meropenem	0.05–0.10	0.05	0.05
	Imipenem	0.78–1.56	1.56	1.56
	Arbekacin	1.56–3.13	1.56	3.13
Bacteroides fragilis (17)	SM-17466	0.05–0.20	0.05	0.20
	Meropenem	0.10–0.78	0.20	0.39
	Imipenem	0.10–0.39	0.39	0.39
	Arbekacin	>100	>100	>100

[a] 50% and 90%, MICs at which 50 and 90% of isolates are inhibited.
[b] Seven strains of *Proteus vulgaris* and eight strains of *Proteus mirabilis*.

TABLE 2. Bactericidal activity of SM-17466 against clinical isolates of methicillin-resistant *S. aureus*[a]

Antibiotic (MIC [μg/ml])	No. of strains with an MBC/MIC ratio of:			
	1	2	4	≥8
SM-17466 (1.56–3.13)	3	10	2	0
Vancomycin (0.39–1.56)	9	6	0	0
Arbekacin (0.78–50)	2	8	2	3
Meropenem (25–50)	5	10	0	0

[a] Fifteen isolates of homogeneous resistant *S. aureus*.

sistant cells could grow on agar containing 12.5 μg of SM-17466 per ml with an inoculum of 10^9 CFU.

Binding to PBPs. Table 3 shows the concentrations of SM-17466, meropenem, and imipenem required to inhibit the binding of [^{14}C]benzylpenicillin to a PBP by 50% (IC_{50}) for methicillin-susceptible *S. aureus* FDA209P and methicillin-resistant MS9408-6H. SM-17466 bound to PBPs 1 to 4 in methicillin-susceptible *S. aureus* at IC_{50}s of <1 μg/ml, with PBPs 2 and 3 showing affinities equivalent to that exhibited with imipenem. The binding affinities of SM-17466 to PBPs 1 and 4, however, were lower by 1 order of magnitude than those of imipenem. In addition, SM-17466 also showed exceedingly high binding (IC_{50}, 5.9 μg/ml) to PBP 2′ in a methicillin-resistant strain. This affinity was 10 to 20 times higher than the values for the other two carbapenems. Against methicillin-resistant *S. aureus*, the three carbapenems had IC_{50}s for PBP 2′ comparable to their antibacterial activities.

In vivo antistaphylococcal activity. The protective effects of SM-17466 against methicillin-susceptible and -resistant *S. aureus* peritoneal infections in mice were determined (Table 4). The numbers of organisms in the intraperitoneal cavity at the point that the antibiotic was administered were 20% ± 5%, 10% ± 4%, and 30% ± 8% (n = 5) of the size of the initial inoculum of *S. aureus* Smith, SM7551, and SM5040-2, respectively. At this point, we observed much bleeding in the intraperitoneal cavity in all mice tested. The 50% effective dose of SM-17466 against the methicillin-susceptible strain Smith was 0.021 mg/kg. This activity was twice that of imipenem-cilastatin and was much greater than the activities of vancomycin and arbekacin. The efficacy of SM-17466 against infection by methicillin-resistant *S. aureus* was equal to those of vancomycin and arbekacin for one strain and one-third that of vancomycin for the second strain. This activity was consistent with the in vitro activity against methicillin-resistant strains. In this experimental infection model, imipenem-cilastatin showed no in vivo efficacy against methicillin-resistant *S. aureus* infections in mice (50% effective dose, >625 mg/kg).

Pharmacokinetic parameters. The pharmacokinetics of SM-17466 were studied with mice that had received a subcutaneous injection of the agent (Table 5). The serum elimination (half-life) of SM-17466 was about 18 min, intermediate between the values for vancomycin and arbekacin. The comparatively high maximum concentration of SM-17466 in serum would contribute to its high area under the concentration-time curve.

Stability against DHP-I. The stability of SM-17466 against swine renal DHP-I was compared with those of meropenem, imipenem, and panipenem. SM-17466 showed resistance to DHP-I hydrolysis almost equal to that of meropenem. The relative rates of hydrolysis were 1.1, 1.0, 4.6, and 2.4 for SM-17466, meropenem, imipenem, and panipenem, respectively.

DISCUSSION

This susceptibility study with a large number of recent clinical isolates revealed that gram-positive organisms were susceptible to SM-17466 in vitro. SM-17466 had potent activity against gram-positive cocci, including methicillin-resistant staphylococci, which was higher than the activities of the other carbapenems, meropenem and imipenem. Of the carbapenems, SM-17466 also had the best activity against penicillin-sensitive and -resistant *S. pneumoniae*. In addition, SM-17466 had good activity against enterococci, especially against *E. faecalis*. It would be of great interest to determine the activity of SM-17466 against vancomycin-resistant enterococci; however, this was not possible because no vancomycin-resistant strains were available. Against gram-negative organisms, SM-17466 exhibited less activity, showing lower activity than imipenem against *S. marcescens* and *P. aeruginosa*, although it showed the same activity as imipenem against *E. coli*, *K. pneumoniae*, and organisms of the *Proteus* family.

SM-17466 bound to normal PBPs in methicillin-susceptible *S. aureus*, showing IC_{50}s of less than 1 μg/ml. SM-17466 also showed excellent binding to PBP 2′ in a methicillin-resistant strain. This affinity, which was 10 to 20 times greater than those shown by PBP 2′ for meropenem and imipenem, was reflected in the good activity of SM-17466 against methicillin-resistant *S. aureus*. Several new β-lactams with high activity against methicillin-resistant staphylococci have been reported (7, 11, 21), and more recently, a novel carbapenem, L-695,256, also showing high activity against methicillin-resistant *S. aureus*, has been reported (6, 8). SM-17466 differs from L-695,256 in that SM-17466 has a 1β-methyl moiety and 2-sulfur connected side chain, although the compounds are similar in terms of having aromatic and quaternary ammonium groups in the C-2 side chain. All these new antibiotics commonly showed strong binding to PBP 2′ compared with β-lactams that show less activity against these resistant organisms. These results indicate that high PBP 2′ binding affinity is necessary for an antibiotic to exhibit potent activity against methicillin-resistant *S. aureus.*

SM-17466 had excellent protective effects against mouse peritoneal infections caused by both methicillin-susceptible *S. aureus* and methicillin-resistant *S. aureus*. SM-17466 had the greatest potency of any agent tested against infections with methicillin-susceptible strains. In infections caused by methi-

TABLE 3. Affinities of PBPs in *S. aureus* FDA209P and MS9408-6H for SM-17466, determined by competition assay with [^{14}C]benzylpenicillin

Antibiotic	IC_{50} (μg/ml) for PBP:					MIC (μg/ml)	
	1	2	2′	3	4	FDA209P	MS9408-6H
SM-17466	0.27	0.25	5.9	0.27	0.16	≤0.006	1.56
Meropenem[a]	0.064	0.45	64	>100	0.053	0.05	50
Imipenem[a]	0.033	0.17	120	0.12	0.0092	0.013	100

[a] Some of the data are from reference 18.

TABLE 4. In vivo antibacterial activities of SM-17466 against *S. aureus* intraperitoneal infections in mice[a]

S. aureus strain (CFU/mouse [multiple of LD_{50}])	Antibiotic	MIC (μg/ml)	ED_{50}	
			mg/kg	95% confidence interval
Smith[b] (3.4×10^7 [47])	SM-17466	0.006	0.021	0.011–0.039
	Vancomycin	1.56	1.8	1.3–9.0
	Arbekacin	0.20	0.42	0.19–0.67
	Imipenem-cilastatin	0.003	0.055	0.031–0.089
SM7551[c] (1.8×10^9 [3])	SM-17466	3.13	8.0	2.5–20.4
	Vancomycin	1.56	9.5	5.2–17.6
	Arbekacin	0.78	5.0	2.2–8.7
	Imipenem-cilastatin	100	>625	
SM5040-2[c] (1.0×10^9 [8])	SM-17466	3.13	25	9.8–47
	Vancomycin	1.56	8.1	4.4–15

[a] Mice received a single subcutaneous dose of the antibiotic 2 h after bacterial challenge. LD_{50}, 50% lethal dose; ED_{50}, 50% effective dose.
[b] Methicillin-susceptible strain.
[c] Homogeneous methicillin-resistant strain.

cillin-resistant strains, the efficacy of SM-17466 was almost the same as that of arbekacin and one-third or equivalent to that of vancomycin. This activity of SM-17466 was consistent with its in vitro activity against methicillin-resistant strains. The stability of SM-17466 in the presence of mouse DHP-I was equivalent to that of meropenem (4). The coadministration of cilastatin with SM-17466 extended the serum half-life of this antibiotic in mice (about 27 min). Our previous study showed that the resistance of carbapenems to hydrolysis by renal DHP-I varied considerably with the animal source of the enzyme (4). It is possible, therefore, that the in vivo activity of SM-17466 could be greater were SM-17466 to be evaluated in experimental animal models in which SM-17466 shows greater resistance to hydrolysis by DHP-I.

A comparative study of the resistance of carbapenems to DHP-I hydrolysis revealed that SM-17466 was quite stable in the presence of swine renal DHP-I. This stability was almost equivalent to that of meropenem and greater than those of imipenem and panipenem (the latter two agents have no 1β-methyl substituent). In a previous study (4), we noted that swine DHP-I, whose substrate specificity was determined against various carbapenem analogs, had a profile similar to that of human DHP-I. It is conceivable, therefore, that SM-17466 may show stability against human DHP-I.

In a preliminary study of acute toxicity, we found that the intravenous administration of 2 g of SM-17466 per kg caused no mortality in mice (*n*, 5), a finding similar to the finding for meropenem (9). In a nephrotoxicity study, there was no alteration in histopathological findings in rabbits when SM-17466 was given at a dose of 150 mg/kg intravenously. With the same dose of imipenem, however, acute proximal tubular necrosis, hyaline droplet formation in proximal tubular epithelial cells, and intratubular proteinaceous casts were observed. In addition, the neurotoxicity of SM-17466 given intracerebroventricularly in mice was shown to be less than that of imipenem (20).

These results justify additional studies to determine the clinical usefulness of SM-17466. As an antibacterial agent that has potential clinical applications against gram-positive organisms, particularly methicillin-resistant *S. aureus*, this carbapenem warrants clinical studies.

TABLE 5. Pharmacokinetic parameters of SM-17466 in mice[a]

Antibiotic[b]	C_{max}[c] (μg/ml)	$t_{1/2}$ (min)	$AUC_{0-\infty}$ (μg · min/ml)	V (ml/kg)
SM-17466	53.9	17.9	1,912	338
Vancomycin	32.9	21.8	1,223	645
Arbekacin	28.5	15.5	1,099	509

[a] Three mice in each group were injected subcutaneously. C_{max}, maximum concentration of the drug in serum; $t_{1/2}$, half-life; $AUC_{0-\infty}$, area under the concentration-time curve from 0 h to infinity; V, volume of distribution.
[b] 25 mg/kg.
[c] 15 min after injection.

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