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Comparative In Vitro Activity of SM7338, a New Carbapenem Antimicrobial Agent

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The comparative in vitro activity of SM7338 was tested against 670 routine clinical isolates and 130 cefoperazone-resistant isolates of bacteria by agar dilution methods. SM7338 was at least as active as imipenem against gram-negative organisms but was slightly less active against gram-positive organisms. SM7338 was particularly active against members of the family *Enterobacteriaceae*, with MICs for 90% of strains of ≤0.125 μg/ml for all species tested. Differences in activity between SM7338 and imipenem were particularly striking against *Proteus vulgaris*, *Proteus mirabilis*, and *Morganella morganii*, against which MICs of SM7338 and imipenem for 90% of strains were 0.125 and 4 μg/ml, respectively. The presence of unique plasmid-mediated β-lactamases in *Pseudomonas aeruginosa* PU21 transconjugants did not affect activity substantially, except in the case of OXA-2 (eightfold-increased MIC) and OXA-3 (fourfold-increased MIC). SM7338 was also active against a laboratory-derived strain of *P. aeruginosa* which hyperproduced chromosomal β-lactamase, inhibiting both the wild type and the mutant at a concentration of 1.0 μg/ml.

SM7338 is a recently developed carbapenem antimicrobial agent that is resistant to hydrolysis by dehydropeptidase I (M. Sungawa, H. Matsumura, T. Inoue, M. Fukasawa, and M. Kato, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 752, 1986). Preliminary data show that the drug is active against a broad range of organisms (A. M. Clarke and S. J. V. Zemcov, 28th ICAAC, abstr. no. 598, 1988). The present study examined the comparative in vitro activity of SM7338 against 670 routine clinical isolates of bacteria. In addition, activity against 130 cefoperazone-resistant organisms was tested. The effect of specific β-lactamases on the activity of the new drug with and without the addition of β-lactamase-inhibiting agents was evaluated with a series of isogenic *Pseudomonas aeruginosa* strains each containing a plasmid which codes for a different unique β-lactamase as well as a mutant *P. aeruginosa* strain with noninducible hyperproduction of β-lactamase. Given the limited utility of beta-lactam antibiotics as single agents for the treatment of serious enterococcal infections, the bactericidal activity of the new agent alone and in combination with gentamicin was also evaluated against several recent clinical isolates of *Enterococcus faecalis*, including multiply resistant strains.

MATERIALS AND METHODS

Organisms. Most bacterial strains used in the study were clinical isolates collected at New England Deaconess Hospital and Massachusetts General Hospital, Boston, Mass. Penicillin-resistant pneumococci and viridans group streptococci were obtained in South Africa as previously described (3). The four strains of β-lactamase-producing *E. faecalis* with high-level gentamicin resistance were recovered at The Childrens Hospital, Boston, Mass. (E. Rhinehart, C. Wennersten, E. Gorss, G. Eliopoulos, R. Moellering, Jr., N. Smith, and D. Goldmann, 28th ICAAC, abstr. no. 1073, 1988). The influence of unique plasmid-mediated β-lactamases on the activity of SM7338 was studied with a series of laboratory transconjugants which were derived from *P. aeruginosa* PU21 and which were isogenic except for plasmids encoding specific β-lactamases (2, 5, 7, 8). These organisms were kindly provided by G. A. Jacoby, Massachusetts General Hospital, Boston. The new antimicrobial agent was also tested against a mutant *P. aeruginosa* strain which produced an increased amount of chromosomal β-lactamase in a noninducible fashion and which was prepared in our laboratory from *P. aeruginosa* PAO38.

Antimicrobial agents. Standard antimicrobial reference powders were obtained from the following sources: SM7338, ICI Pharmaceuticals Group, Wilmington, Del.; ceftazidime, Eli Lilly & Co., Indianapolis, Ind.; imipenem, Merck Sharp & Dohme Research Laboratories, Rahway, N.J.; metronidazole, Searle Laboratories, Skokie, Ill.; tazobactam, Lederle Laboratories, Pearl River, N.Y.; potassium clavulanate, Beecham Laboratories, Bristol, Tenn.; sulbactam, Pfizer Inc., Groton, Conn.; and gentamicin sulfate, Elkins-Sinn, Inc., Cherry Hill, N.J.

Susceptibility studies. MICs were determined by an agar dilution method (6). Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) was used in testing aerobes and facultative organisms. It was supplemented with 5% defibrinated sheep blood when used in testing nonenterococcal streptococci. *Campylobacter jejuni* was tested on brucella agar (BBL) with 10% defibrinated sheep blood. Wilkins-Chalgren agar (Oxoid Ltd., Basingstoke, Hampshire, England) was used in testing anaerobes. It was supplemented with 5% defibrinated sheep blood when used in testing gram-positive cocci and *Bacteroides melaninogenicus*.

Bacterial suspensions were prepared in Mueller-Hinton broth (BBL) from fresh overnight cultures and applied to plates with a 32-prong inoculator, yielding a final inoculum of ca. 10^4 CFU per spot. Plates containing aerobic or facultative organisms were examined after 16 to 20 h of incubation in room air at 35°C. Plates with *C. jejuni* were evaluated after

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TABLE 1. Comparative in vitro activity of SM7338 against routine clinical isolates

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Enterococcus faecalis (30)[b]	SM7338	4–8	4	8
	Imipenem	1–4	2	2
	Ciprofloxacin	0.5–2	1	2
Enterococcus faecium (10)	SM7338	16–64	64	64
	Imipenem	4–32	16	32
	Ciprofloxacin	0.5–8	4	4
Enterococcus avium (10)	SM7338	2–64	2	4
	Imipenem	0.25–4	0.25	0.25
	Ciprofloxacin	2	2	2
Listeria monocytogenes (10)	SM7338	0.06–0.25	0.125	0.25
	Imipenem	0.06–0.25	0.25	0.25
	Ciprofloxacin	2	2	2
Staphylococcus aureus, methicillin susceptible (15)	SM7338	0.125–0.25	0.125	0.25
	Imipenem	0.015–0.03	0.03	0.03
	Ciprofloxacin	0.5	0.5	0.5
Staphylococcus aureus, methicillin resistant (15)	SM7338	0.5–32	16	32
	Imipenem	0.25–16	4	16
	Ciprofloxacin	0.5–1	0.5	0.5
Staphylococcus epidermidis, methicillin susceptible (17)	SM7338	0.06–4	0.25	4
	Imipenem	0.015–32	0.06	2
	Ciprofloxacin	0.25–0.5	0.25	0.5
Staphylococcus epidermidis, methicillin resistant (41)	SM7338	0.03–16	4	16
	Imipenem	0.015–32	0.5	16
	Ciprofloxacin	0.25–1	0.25	0.5
Streptococcus group A (10), B (10), C (5), and G (5)	SM7338	0.008–0.125	0.03	0.125
	Imipenem	0.008–0.06	0.015	0.03
	Ciprofloxacin	0.5–2	1	1
Streptococcus pneumoniae, penicillin susceptible (10)	SM7338	0.015–0.03	0.015	0.03
	Imipenem	0.015–0.06	0.015	0.015
	Ciprofloxacin	1–2	1	2
Streptococcus pneumoniae, penicillin resistant (20)	SM7338	0.03–2	0.06	2
	Imipenem	0.015–2	0.06	1
	Ciprofloxacin	1–4	1	2
Streptococci, viridans group, penicillin resistant (20)	SM7338	0.015–0.25	0.06	0.25
	Imipenem	0.015–0.25	0.03	0.0125
	Ciprofloxacin	0.5–4	1	1
Streptococci, viridans group, penicillin resistant (10)	SM7338	0.25–2	2	4
	Imipenem	0.25–2	0.5	2
	Ciprofloxacin	1–8	2	4
Aeromonas hydrophila (10)	SM7338	0.03–0.5	0.06	0.25
	Imipenem	0.25–16	1	8
	Ciprofloxacin	0.008–0.06	0.03	0.06
Acinetobacter anitratus (10)	SM7338	0.25–1	0.5	1
	Imipenem	0.25–1	0.5	0.5
	Ciprofloxacin	0.25–0.5	0.25	0.25
Campylobacter jejuni (13)	SM7338	0.004–0.015	0.008	0.015
	Imipenem	0.008–0.06	0.03	0.03
	Ciprofloxacin	0.125–1	0.5	1
Citrobacter freundii (25)	SM7338	0.015–0.125	0.03	0.06
	Imipenem	0.25–2	0.5	1
	Ciprofloxacin	0.015–0.125	0.015	0.06

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TABLE 1—*Continued*

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Enterobacter aerogenes (25)	SM7338	0.03–0.06	0.03	0.06
	Imipenem	0.125–1	0.5	1
	Ciprofloxacin	0.03–0.06	0.06	0.06
Enterobacter cloacae (40)	SM7338	0.015–0.06	0.03	0.06
	Imipenem	0.25–1	0.25	0.25
	Ciprofloxacin	0.015–0.25	0.03	0.125
Escherichia coli (40)	SM7338	0.015–0.03	0.03	0.03
	Imipenem	0.06–0.25	0.125	0.125
	Ciprofloxacin	0.008–0.06	0.015	0.03
Klebsiella pneumoniae (35)	SM7338	0.03–0.06	0.06	0.06
	Imipenem	0.125–0.5	0.25	0.25
	Ciprofloxacin	0.06–0.5	0.06	0.06
Morganella morganii (30)	SM7338	0.06–0.25	0.125	0.125
	Imipenem	1–4	4	4
	Ciprofloxacin	0.015–0.25	0.03	0.03
Proteus mirabilis (30)	SM7338	0.03–0.25	0.06	0.125
	Imipenem	0.25–4	1	4
	Ciprofloxacin	0.03–0.25	0.06	0.25
Proteus vulgaris (20)	SM7338	0.06–0.25	0.125	0.125
	Imipenem	1–8	2	4
	Ciprofloxacin	0.03–0.125	0.03	0.125
Providencia rettgeri (9)	SM7338	0.06–0.125	0.125	
	Imipenem	0.5–2	2	
	Ciprofloxacin	0.06–2	0.06	
Pseudomonas aeruginosa (40)	SM7338	0.06–4	0.25	2
	Imipenem	1–32	2	4
	Ciprofloxacin	0.03–0.5	0.25	0.5
Pseudomonas cepacia (10)	SM7338	0.125–32	0.5	32
	Imipenem	0.125–16	0.5	16
	Ciprofloxacin	0.125–1	0.25	1
Pseudomonas maltophilia (10)	SM7338	16–128	64	128
	Imipenem	128	128	128
	Ciprofloxacin	0.5–2	1	1
Serratia marcescens (20)	SM7338	0.03–0.5	0.06	0.06
	Imipenem	0.25–1	0.5	1
	Ciprofloxacin	0.06–2	0.125	0.125
Anaerobic gram-positive cocci[c] (15)	SM7338	0.008–0.5	0.125	0.25
	Imipenem	0.008–0.5	0.125	0.5
	Metronidazole	0.25–128	0.25	128
Bacteroides fragilis (10)	SM7338	0.125–2	0.125	1
	Imipenem	0.06–2	0.06	2
	Metronidazole	0.25–0.5	0.5	0.5
Bacteroides melaninogenicus (8)	SM7338	0.03–0.06	0.06	
	Imipenem	0.03–0.125	0.06	
	Metronidazole	0.25–0.25	0.25	
Clostridium perfringens (8)	SM7338	0.008–0.03	0.015	
	Imipenem	0.03–0.06	0.06	
	Metronidazole	0.5–1	1	

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively.
[b] Includes four β-lactamase-producing high-level-gentamicin-resistant isolates.
[c] *Peptostreptococcus* (n = 5) and *Peptococcus* (n = 10) spp.

TABLE 2. Comparative in vitro activity of SM7338 against selected cefoperazone-resistant isolates

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Acinetobacter anitratus (20)	SM7338	0.5–2	0.5	1
	Imipenem	0.25–0.5	0.5	0.5
	Ciprofloxacin	0.125–2	0.5	2
Citroboacter freundii (15)	SM7338	0.015–0.125	0.06	0.125
	Imipenem	0.125–2	1	1
	Ciprofloxacin	0.03–1	0.06	0.125
Enterobacter cloacae (20)	SM7338	0.25–8	0.5	4
	Imipenem	0.5–8	4	8
	Ciprofloxacin	0.125–4	0.25	0.25
Escherichia coli (6)	SM7338	0.03–0.5	0.06	
	Imipenem	0.25–1	0.5	
	Ciprofloxacin	0.03–0.25	0.03	
Klebsiella oxytoca (10)	SM7338	0.125–0.5	0.25	0.25
	Imipenem	1–4	1	4
	Ciprofloxacin	0.125–0.25	0.125	0.5
Klebsiella pneumoniae (6)	SM7338	0.03–0.06	0.06	
	Imipenem	0.25–1	0.25	
	Ciprofloxacin	0.06–0.125	0.125	
Pseudomonas aeruginosa (20)	SM7338	2–64	8	32
	Imipenem	1–128	8	64
	Ciprofloxacin	0.125–32	1	2
Pseudomonas maltophilia (20)	SM7338	128–>128	>128	>128
	Imipenem	>128	>128	>128
	Ciprofloxacin	4–>128	8	>128
Bacteroides fragilis (16)	SM7338	0.125–0.25	0.125	0.25
	Imipenem	0.06–2	0.06	0.25
	Metronidazole	0.25–1	0.5	1

[a] See Table 1, footnote *a*.

24 h of incubation in a microaerophilic atmosphere (Campypak; BBL) at 35°C. Anaerobic plates were examined after 48 h of incubation at 35°C in an anaerobic environment (GasPak; BBL), except for *Clostridium perfringens* plates, which were examined after 24 h of incubation.

Bactericidal activity against enterococci. Time kill curve techniques (4) were used to examine possible bactericidal synergism against *E. faecalis*. In glucose phosphate broth (BBL) alone or supplemented with 50% human serum, 10 μg of SM7338 per ml (a concentration equal to more than twice the MIC for each strain) was combined with 5 μg of gentamicin per ml (a subinhibitory concentration) and tested against *E. faecalis* strains lacking high-level gentamicin resistance. Bactericidal synergism was defined as a 100-fold decrease in CFU per milliliter at 24 h of incubation by the combination as compared with SM7338 alone. Concentrations of 10 and 20 μg of SM7338 per ml were tested in broth alone or supplemented with 50% human serum against β-lactamase-producing *E. faecalis* strains with high-level gentamicin resistance. The stability of SM7388 at concentrations of 80 and 20 μg/ml over 24 h of incubation at 35°C in broth supplemented with 50% serum was determined by a microbiological bioassay (1).

RESULTS

Susceptibility of routine pathogens. SM7338 showed excellent activity against a broad variety of routine clinical isolates, including gram-positive and gram-negative aerobes, as well as anaerobes (Table 1). SM7338 was at least as active as imipenem against all the gram-negative organisms tested and was markedly more active against several species, including *Aeromonas hydrophila*, *Morganella morganii*, *Proteus* spp., and *Serratia marcescens*. The new carbapenem provided no advantage over imipenem against *Pseudomonas cepacia* and *Pseudomonas maltophilia*. The activity of SM7338 was similar to that of ciprofloxacin against most members of the family *Enterobacteriaceae*. SM7338 was highly active against beta-lactam-susceptible gram-positive organisms, although it was often two- to fourfold less active than was imipenem. Penicillin-resistant viridans group streptococci and *Streptococcus pneumoniae* and methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* were less susceptible to both carbapenems than were their beta-lactam-susceptible counterparts. The new drug was considerably less active than was imipenem against *E. faecalis* and *Enterococcus avium*. SM7338 was at least as active as imipenem against anaerobic organisms.

Susceptibility of cefoperazone-resistant organisms. SM7338 displayed a high degree of activity against many of the cefoperazone-resistant organisms tested (Table 2). However, cefoperazone-resistant *Enterobacter cloacae* and *P. aeruginosa* isolates were markedly less susceptible to SM7338 and imipenem than were the unselected isolates.

Influence of β-lactamases on activity. Of the single plasmid-mediated β-lactamases expressed in the *P. aeruginosa* PU21 transconjugants, only OXA-2 (MIC, 4 μg/ml) and OXA-3 (MIC, 8 μg/ml) resulted in SM7338 activity that was significantly different from that against the parent strain (MIC, 1 μg/ml). Because synergistic activity of imipenem combined with β-lactamase inhibitors has been demonstrated against imipenem-resistant *P. aeruginosa* strains (M. D. Zitkis, F. W. Goldstein, J. W. Acar, and L. Gutmann, 28th ICAAC, abstr. no. 89, 1988), we examined the effect of β-lactamase inhibitors on the activity of SM7338 against these strains. The addition of tazobactam (10 μg/ml) decreased the MIC against strains bearing OXA-2 (MIC, 1 μg/ml) and OXA-3 (MIC, 2 μg/ml). The addition of either sulbactam or clavulanate had no significant impact. β-Lactamases other than OXA-2 and OXA-3 did not affect the MIC of SM7338 against plasmid-containing strains as compared with that against the plasmid-free parent strain. These included TEM-1, TEM-2, PSE-1, PSE-2, PSE-3, PSE-4, CARB-4, OXA-4, OXA-5, and OXA-6. The MIC for neither PU21 nor the remaining derivatives was affected by the addition of a β-lactamase inhibitor. The MIC of SM7338 against the derepressed strain of *P. aeruginosa* that hyperproduced β-lactamase was identical to that against the parent strain (1 μg/ml). The MICs of ceftazidime were 2 μg/ml against the parent strain and 32 μg/ml against the mutant.

Bactericidal activity against enterococci. The combination of SM7338 and gentamicin displayed bactericidal synergism against five of six strains of routine (non-high-level-gentamicin-resistant) *E. faecalis* isolates tested in broth alone. The magnitude of enhanced killing by the combination relative to the carbapenem alone averaged 3.4 $\log_{10}$ CFU/ml. The sixth strain was killed by SM7338 alone, so that the definition of bactericidal synergism could not be met. Preliminary experiments excluded significant antibiotic carryover. Against high-level-gentamicin-resistant β-lactamase-producing strains of *E. faecalis*, SM7338 at 10 and 20 μg/ml resulted in 4.5 and 1.8 $\log_{10}$ residual CFU/ml, respectively, after 24 h of incubation in broth. However, when these experiments were repeated with 50% human serum, cultures demonstrated substantial regrowth after initial killing. This result could be attributed to the loss of drug activity in this medium. Ninety percent of the microbiological activity of SM7338 was lost after 24 h when the drug was incubated with 50% human serum in room air at 35°C. The drug was much more stable in glucose phosphate broth alone, with essentially no loss of activity in 24 h.

DISCUSSION

The present study confirmed the excellent activity of SM7338 against a wide variety of bacteria. The activity of the new drug against most gram-negative organisms was superior or comparable to that of imipenem. SM7338 was superior to imipenem against *M. morganii*, *Proteus* spp., and *S. marcescens*. The newer compound offered no advantage over imipenem against gram-positive organisms. The gaps in the activity of SM7338 were similar to those of imipenem; *Enterococcus faecium*, *P. maltophilia*, *P. cepacia*, and cefoperazone-resistant *P. aeruginosa* were relatively resistant to the new drug. Activity was not affected by most plasmid-mediated β-lactamases or by hyperproduction of a chromosomal β-lactamase, as is the case for imipenem (9). Data that emerged from our time kill curve studies with *E. faecalis* suggesting a substantial loss of activity of SM7338 in serum will be an important consideration in future studies.

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LITERATURE CITED

1. **Anhalt, J. P.** 1985. Assays for antimicrobial agents in body fluids, p. 1009–1014. *In* E. H. Lenette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
2. **Calderwood, S. B., A. Gardella, A. M. Philippon, G. A. Jacoby, and R. C. Moellering, Jr.** 1982. Effects of azlocillin in combination with clavulanic acid, sulbactam, and *N*-formimidoyl thienamycin against β-lactamase-producing, carbenicillin-resistant *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **22:** 266–271.
3. **Eliopoulos, G. M., A. Gardella, and R. C. Moellering, Jr.** 1982. In vitro activity of Sch 29482 in comparison with other oral antibiotics. J. Antimicrob. Chemother. **9**(Suppl. C):143–152.
4. **Eliopoulos, G. M., and R. C. Moellering, Jr.** 1981. Susceptibility of enterococci and *Listeria monocytogenes* to *N*-formimidoyl thienamycin alone and in combination with an aminoglycoside. Antimicrob. Agents Chemother. **19:**789–793.
5. **Medeiros, A. A., M. Cohenford, and G. A. Jacoby.** 1985. Five novel plasmid-determined β-lactamases. Antimicrob. Agents Chemother. **27:**715–719.
6. **National Committee for Clinical Laboratory Standards.** 1988. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Tentative standard. National Committee for Clinical Laboratory Standards publication M7-T2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
7. **Philippon, A. M., G. C. Paul, and G. A. Jacoby.** 1986. New plasmid-mediated oxacillin-hydrolyzing β-lactamase in *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. **17:**415–422.
8. **Philippon, A. M., G. C. Paul, A. P. Thabaut, and G. A. Jacoby.** 1986. Properties of a novel carbenicillin-hydrolyzing β-lactamase (CARB-4) specified by an IncP-2 plasmid from *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **29:**519–520.
9. **Sanders, C. C., and W. E. Sanders, Jr.** 1986. Type I β-lactamases of gram-negative bacteria: interactions with β-lactam antibiotics. J. Infect. Dis. **154:**792–800.