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Antimicrobial and β-Lactamase Inhibitory Activities of Carpetimycins A and B, New Carbapenem Antibiotics

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Carpetimycins A and B showed widely broad spectra and potent activity against gram-positive and gram-negative bacteria, including various species of anaerobic bacteria. The antimicrobial activity of carpetimycin A was 8 to 64 times greater than that of carpetimycin B and 4 to 128 times greater than that of cefoxitin. The inhibitory concentration of carpetimycin A required to inhibit more than 90% of clinical isolates was 0.39 μg/ml for *Escherichia coli* and *Klebsiella* and 1.56 μg/ml for *Proteus* and *Staphylococcus aureus*. At a concentration of 3.13 μg/ml, carpetimycin A inhibited almost all clinical isolates of *Enterobacter* and *Citrobacter*, which showed resistance to many clinically used β-lactam antibiotics. Carpetimycins A and B furthermore were shown to have potent inhibitory activities against several kinds of β-lactamases produced by β-lactam-resistant strains; they inhibited not only penicillinase-type β-lactamases but also cephalosporinase-type β-lactamases, which were insensitive to clavulanic acid. In combination with β-lactam antibiotics such as ampicillin, carbenicillin, and cefazolin, carpetimycins A and B showed synergistic activities against β-lactam-resistant bacteria.

Carpetimycins (CPMs) A and B (12; T. Mori, M. Nakayama, A. Iwasaki, S. Kimura, T. Mizoguchi, S. Tanabe, A. Murakami, I. Watanabe, M. Okuchi, H. Ito, Y. Saino, and F. Kobayashi, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 165, 1980) were isolated from a broth filtrate of *Streptomyces* sp. KC-6643 by several adsorption chromatography steps and preparative high-performance liquid chromatography.

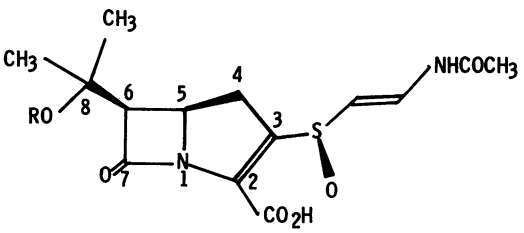
The structures of carpetimycins A and B (13) were determined to be a family of carbapenem antibiotics (Fig. 1), as are thienamycin (1), olivanic acid derivatives (3–5), PS-5 (22), and asparenomycin A (20). CPMs A and B showed considerable stability in aqueous solution at pH 6.0 and 7.8 and showed potent antimicrobial activities against gram-positive and gram-negative bacteria, including β-lactamase-producing strains. The present paper deals with the biological properties of CPMs A and B.

MATERIALS AND METHODS

Antibiotics. CPMs A and B were prepared by Tokyo Research Laboratories, Kowa Co., Ltd., Tokyo, Japan. Ampicillin (APC), cefoxitin (CFX), cefazolin (CEZ), carbenicillin (CBC), cephaloridine (CER), and penicillin G (PCG) were obtained commercially. Clavulanic acid (CVA) was provided from Beecham Yakuhin Co., Ltd., Tokyo, Japan.

Test strains. The type strains used for these experiments were stocked in our laboratories. β-Lactamase-producing strains and clinical isolates of various species of bacteria were provided by several university hospitals in Tokyo. These facultative bacteria were kept in semisolid nutrient agar or the same medium supplemented with 10% horse blood until use. Anaerobic bacteria were kept in semisolid GAM agar (Nissui Pharmaceutical Co., Ltd., Tokyo).

Determination of MICs. The minimum inhibitory concentrations (MICs) of antibiotics were determined by the usual twofold serial dilution technique. Facultative bacteria were subcultured in tryptic soy broth (Eiken Chemical Co., Ltd., Tokyo) and were tested with heart infusion agar (Eiken Chemical Co., Ltd.) or



R : H Carpetimycin A

R : SO_3H Carpetimycin B

FIG. 1. Chemical structures of CPMs A and B.

heart infusion broth (Difco Laboratories, Detroit, Mich.). For *Streptococcus*, *Corynebacterium*, *Bordetella*, and *Haemophilus*, heart infusion agar supplemented with 10% horse blood was used. Anaerobic bacteria were subcultured in GAM broth (Nissui Pharmaceutical Co., Ltd.), and GAM agar was used for testing. One loopful (5 μl) of diluted overnight culture of each test organism (10^6 colony-forming units [CFU] per ml) was inoculated onto assay media containing graded concentrations of the test antibiotic, and the organisms were cultured at 37°C for 18 h. A GasPak jar (BBL Microbiology Systems, Cockeysville, Md.) was used for culturing anaerobes.

Effects of several factors on antimicrobial activity. Inoculum size was varied by adding a drop (40 μl) of several 10-fold dilutions of overnight culture of *Staphylococcus aureus* and *Escherichia coli* to 2 ml of heart infusion broth containing antibiotic. The pH of the broth was varied by adding 1 N NCl or 1 N NaOH to achieve the desired initial pH value. Sterile horse serum was added directly to broth to study the effect of serum on antimicrobial activity. After the determination of MICs by means of a standard twofold dilution method, one loopful (5 μl) from tubes containing trace growth or no discernible growth was subcultured on an antibiotic-free nutrient agar (Eiken Chemical Co., Ltd.) plate. The minimum bactericidal concentrations (MBCs) were defined as the lowest concentrations of the drug that yielded less than 10 colonies on subculture.

Bactericidal activity. The bactericidal activity of CPM A was tested against *S. aureus* and *E. coli* in heart infusion broth at 37°C with rocking by a Monod apparatus. Portions of the culture were taken at selected intervals, and viable cells were counted by the usual agar-dilution technique.

Preparation of β-lactamase. β-Lactamases from seven strains were extracted from the culture cells of each strain. The cells were harvested by centrifugation, washed twice with 0.1 M phosphate buffer (pH 7.0),

TABLE 1. Antimicrobial spectra of CPMs A and B, APC, and CFX

Organism[a]	MIC (μg/ml)[b] of:			
	CPM A	CPM B	APC	CFX
Staphylococcus aureus 209P JC-1	0.39	6.25	0.05	1.56
S. aureus Smith	0.78	6.25	0.1	1.56
S. epidermidis ATCC 1228	0.78	6.25	0.78	1.56
Micrococcus luteus ATCC 9341	≤0.025	0.2	≤0.025	0.05
Bacillus subtilis ATCC 6633	0.2	6.25	≤0.025	1.56
Streptococcus pneumoniae IID 552 type 1	0.2	1.56	≤0.025	1.56
S. pyogenes Cook	0.39	6.25	≤0.025	0.78
S. faecalis Hayashi	12.5	>100	0.78	>100
Corynebacterium diphtheriae PW-8-2	0.2	3.13	0.39	1.56
Bordetella pertussis IID 510	0.39	0.78	≤0.025	0.2
Haemophilus influenzae Km-1	0.78	6.25	0.39	6.25
Escherichia coli NIHJ JC-2	0.05	1.56	6.25	6.25
Salmonella typhi H-901	≤0.025	1.56	0.2	0.78
S. paratyphi A 1015	≤0.025	0.78	0.39	1.56
S. paratyphi B 8006	≤0.025	1.56	0.39	1.56
S. typhimurium	0.05	3.13	0.39	1.56
S. enteritidis IID 604	0.1	3.13	0.78	3.13
Shigella dysenteriae IID 633 type 3	≤0.025	0.78	0.39	0.78
S. flexneri 2a	≤0.025	3.13	0.78	3.13
S. sonnei T	0.1	6.25	3.13	3.13
Klebsiella pneumoniae PCI 602	0.2	6.25	12.5	0.78
Enterobacter aerogenes IID 972	0.1	3.13	6.25	>100
E. cloacae IID 977	0.78	6.25	>100	>100
Hafnia alvei IID 978	0.2	6.25	50	6.25
Serratia marcescens NHL	0.2	6.25	50	12.5
Proteus vulgaris IID 874	0.39	12.5	>100	6.25
P. morganii IFO 3168	0.39	12.5	>100	6.25
P. rettgeri IFO 13501	1.56	50	0.2	1.56
P. inconstans Km-115	0.78	12.5	100	6.25
Pseudomonas aeruginosa A3	6.25	50	>100	>100
P. aeruginosa NCTC 10490	6.25	25	100	>100
P. cepacia IID 1340	50	50	>100	>100
P. maltophilia IID 1275	>100	>100	>100	>100
P. putida IID 5121	1.56	100	>100	>100
Achromobacter xylosoxidans Km-17	6.25	>100	6.25	100
Acinetobacter calcoaceticus Km-8	1.56	>100	50	>100
Flavobacterium meningosepticum Km-14	>100	>100	25	>100

[a] Inoculum size was one loopful of bacterial suspension (10^6 CFU/ml).
[b] Heart infusion agar was used as a basal medium.

TABLE 2. Antimicrobial activity of CPMs A and B, APC, and CFX against anaerobic bacteria

Organism[a]	MIC (μg/ml)[b] of:			
	CPM A	CPM B	APC	CFX
Eubacterium limosum ATCC 8486	≤0.025	≤0.025	0.2	≤0.025
E. lentum H-1	3.13	50	1.56	3.13
Propionibacterium acnes ATCC 11828	0.05	1.56	0.2	0.2
Fusobacterium varium ATCC 8501	100	100	3.13	50
F. necrophorum FM-8S	0.39	12.5	25	0.78
Peptococcus variabilis ATCC 14955	0.39	6.25	0.39	0.2
P. asaccharolyticus TCH-2	0.05	0.2	0.05	0.1
Veillonella parvula FA-37	0.39	6.25	0.1	0.39
Clostridium sporogenes GM-6248	0.2	0.39	0.2	0.39
Bacteroides fragilis	3.13	12.5	25	25

[a] See Table 1, footnote *a*.
[b] GAM agar was used as a medium.

TABLE 3. Antimicrobial activity of CPMs A and B and selected β-lactam antibiotics against clinical isolates

Organism (no. of strains)	Antibiotic	MIC (μg/ml) of:		
		Range	For 50% inhibition	For 90% inhibition
Staphylococcus aureus (27)	CPM A	0.78–6.25	0.78	1.56
	CPM B	3.13–25	6.25	12.5
	APC	0.1–25	1.56	3.13
	CFX	1.56–12.5	3.13	6.25
	CEZ	0.39–3.13	0.78	1.56
Escherichia coli (27)	CPM A	≦0.025–0.78	0.05	0.39
	CPM B	3.13	3.13	3.13
	APC	3.13–>100	6.25	>100
	CFX	6.25–12.5	6.25	12.5
	CEZ	1.56–50	3.13	12.5
Klebsiella pneumoniae (27)	CPM A	0.05–0.78	0.1	0.39
	CPM B	3.13–6.25	3.13	6.25
	APC	25–>100	50	>100
	CFX	3.13–6.25	6.25	6.25
	CEZ	3.13–>100	3.13	50
Enterobacter spp. (27)	CPM A	0.2–3.13	0.78	3.13
	CPM B	3.13–12.5	6.25	12.5
	APC	100–>100	>100	>100
	CFX	25–>100	>100	>100
	CEZ	25–>100	>100	>100
Citrobacter spp. (27)	CPM A	0.05–6.25	0.2	3.13
	CPM B	1.56–25	3.13	12.5
	APC	1.56–>100	>100	>100
	CFX	25–>100	>100	>100
	CEZ	3.13–>100	>100	>100
Serratia marcescens (27)	CPM A	0.2–50	3.13	12.5
	CPM B	6.25–>100	25	>100
	APC	25–>100	>100	>100
	CFX	6.25–>100	100	>100
	CEZ	>100	>100	>100
Indole-negative *Proteus* spp. (20)	CPM A	0.2–3.13	0.78	1.56
	CPM B	12.5–50	25	25
	APC	1.56–25	1.56	3.13
	CFX	3.13–25	6.25	12.5
	CEZ	6.25–25	12.5	25
Indole-positive *Proteus* spp. (34)	CPM A	≦0.025–1.56	0.78	1.56
	CPM B	1.56–50	25	25
	APC	1.56–>100	>100	>100
	CFX	0.78–>100	6.25	50
	CEZ	1.56–>100	>100	>100

TABLE 4. Antimicrobial activity of CPMs A and B and CFX against APC-resistant strains

Organism[a]	MIC (μg/ml)[b] of:			
	CPM A	CPM B	APC	CFX
Staphylococcus aureus 47	6.25	50	100	12.5
Escherichia coli ML1410 REC1	0.2	3.13	>100	3.13
E. coli 59	0.05	1.56	>100	6.25
E. coli 68	0.05	3.13	>100	6.25
Citrobacter freundii 1	0.78	6.25	>100	>100
C. freundii 9	0.1	3.13	>100	12.5
C. freundii 24	1.56	12.5	>100	>100
Klebsiella pneumoniae 25	0.78	6.25	>100	6.25
K. pneumoniae 32	3.13	12.5	>100	>100
Enterobacter cloacae 3	3.13	12.5	>100	>100
E. cloacae 4	3.13	12.5	>100	>100
Serratia marcescens 4	3.13	25	>100	50
Proteus vulgaris 69	0.78	25	>100	3.13
P. morganii 41	1.56	25	>100	>100
P. inconstans 113	0.39	6.25	50	3.13
P. inconstans 115	0.78	12.5	100	6.25

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

and resuspended in the same buffer. The bacterial cells were disrupted by ultrasonic treatment in an ice bath. The crude extracts were centrifuged for 40 min at 10,000 × *g* at 4°C, and streptomycin (final, 1.5% [wt/vol]) was added to the supernatant for the removal of nucleic acids. The supernatant fluids were then dialyzed overnight against distilled water. β-Lactamases from *E. coli* ML1410 REC1 and *E. coli* 59 were purified by quaternary aminoethyl-Sephadex A-50 column chromatography, and β-lactamases from *E. coli* 68, *Citrobacter freundii* 24, *Proteus morganii* 41, *Pseudomonas aeruginosa* 48, and *Proteus vulgaris* 69 were purified by carboxymethyl-Sephadex C-50 column chromatography by the method described previously (7, 19). The enzymes were further purified by gel filtration with Sephadex G-100.

Assay of β-lactamase inhibitory activity. As substrates, 100 μM final concentrations of PCG (0.1 ml) and CER (0.1 ml) were used for penicillinase (PCase) and for cephalosporinase (CSase) or cefuroximase (CXase), respectively. An inhibitor solution (0.1 ml) was incubated with a partially purified enzyme solution (0.1 ml) at 30°C for 5 min before the addition of substrate. The hydrolysis of PCG was determined by a modification of the Novick microiodometric method (16). The hydrolysis of CER was measured directly by

TABLE 5. Effect of inoculum size, medium pH, and horse serum on the activity of CPM A, CFX, and CEZ

Factor	*S. aureus* 209P JC1						*E. coli* NIHJ JC2					
	CPM A		CFX		CEZ		CPM A		CFX		CEZ	
	MIC[a]	MBC[a]	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Inoculum size (CFU/ml)												
10^4	0.39	0.78	0.78	0.78	0.1	0.1	0.05	0.05	6.25	12.5	1.56	1.56
10^5	0.39	0.78	0.78	1.56	0.1	0.2	0.05	0.1	12.5	12.5	1.56	1.56
10^6	0.39	0.78	1.56	1.56	0.1	0.2	0.05	0.39	12.5	12.5	1.56	3.13
10^7	0.78	1.56	1.56	3.13	0.2	0.39	0.2	0.39	12.5	12.5	1.56	1.56
10^8	0.78	3.13	1.56	3.13	0.2	0.39	0.39	0.78	12.5	25	3.13	3.13
Medium pH												
6.2	0.2	0.39	0.78	1.56	0.1	0.2	0.05	0.1	12.5	12.5	1.56	3.13
7.2	0.39	0.78	1.56	1.56	0.1	0.2	0.05	0.39	12.5	12.5	1.56	3.13
8.2	0.78	6.25	1.56	3.13	0.2	0.39	0.1	0.78	6.25	6.25	1.56	3.13
Horse serum (%)												
0	0.39	0.78	1.56	1.56	0.1	0.2	0.05	0.2	12.5	12.5	1.56	1.56
10	0.39	1.56	1.56	1.56	0.1	0.2	0.1	0.2	6.25	6.25	1.56	1.56
25	0.39	12.5	1.56	1.56	0.1	0.1	0.2	0.39	3.13	6.25	1.56	3.13
50	0.39	25	0.78	1.56	0.1	0.1	0.78	3.13	3.13	6.25	3.13	12.5

[a] MICs and MBCs (micrograms per milliliter) were determined in heart infusion broth (pH 7.2), except for the growth medium experiment. The inoculum size was one loopful of bacterial suspension (10^6 CFU/ml), except for the experiment with inoculum sizes.

the spectrophotometrical method at 255 nm with a double-beam spectrophotometer (type 124, Hitachi Co., Ltd., Tokyo). All reactions were carried out in 50 mM phosphate buffer (pH 7.0; 0.7 ml for PCase and 2.7 ml for CSase or CXase).

One unit of enzyme activity was defined as the amount which hydrolyzed 1 μmol of a substrate per min at 30°C.

The concentration giving 50% inhibition of β-lactamase inhibitor was calculated from a plot of percentage of inhibition against an inhibitor concentration. The Michaelis constant (K_m) and the dissociation constant of enzyme-inhibitor complex (K_i) were determined by the Lineweaver-Burk plot (9) and the Dixon plot (6), respectively.

Assay of synergistic activity. A cell suspension of β-lactam-resistant strain was mixed with melted heart infusion agar containing 100 or 400 μg of β-lactam antibiotic per ml and poured in petri dishes. Thin paper disks (8 mm in diameter, Toyo Roshi Co., Ltd., Tokyo) wetted with 25 μl of 0.1 mg of CPMs A and B per ml were placed on assay agar plates containing the test organism (10^7 CFU/ml). Synergistic activity of CPMs A and B with the other β-lactam antibiotics was judged from the diameters of inhibition zones.

RESULTS

Antimicrobial spectrum. The antimicrobial spectra of CPMs A and B compared with APC and CFX are shown in Table 1. CPMs A and B showed broad-spectrum activities against various species of gram-positive and gram-negative bacteria, including anaerobic bacteria. Against gram-positive bacteria, CPM A was more active than CPM B and CFX, but less active than APC. Against gram-negative bacteria, CPM A was the most active among three antibiotics. Most of the strains tested, except *Pseudomonas* and *Flavobacterium*, were inhibited at a concentration of 1.56 μg/ml or less. The antimicrobial activity of

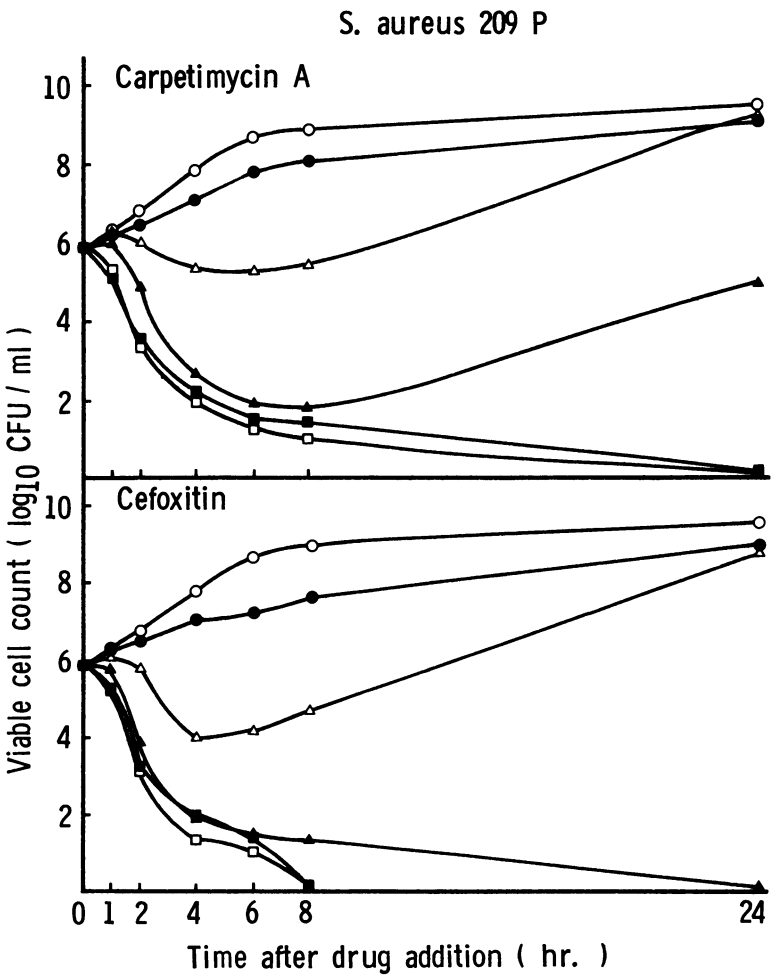


FIG. 2. Bactericidal activity of CPM A and CFX against *E. coli* NIHJ and *S. aureus* 209P. Symbols: ○, control; ●, 0.5 MIC; △, 1 MIC; ▲, 2 MIC; □, 4 MIC; and ■, 8 MIC.

CPM B was almost equal to that of CFX. However, CPMs A and B inhibited APC- or CFX-resistant bacteria, but showed no effect against nonglucose-fermenting gram-negative rods such as *Pseudomonas, Acinetobacter, Achromobacter*, and *Flavobacterium*.

Against all anaerobic bacteria except *Fusobacterium*, CPMs A and B exhibited potent activity (Table 2).

Antimicrobial activity. The in vitro antimicrobial activity of CPMs A and B against clinical isolates was tested by an agar dilution technique (Table 3).

Most of the *S. aureus* isolates were inhibited by 1.56 μg or less of CPM A per ml. The activity of CPM A was similar to that of APC and was more potent than the activities of CPM B and CFX. CPM B was approximately two times less active than CFX and four to eight times less active than CPM A.

CPM A inhibited all strains of *E. coli* and *Klebsiella pneumoniae* at a concentration of 0.78 μg/ml or less, being about 64 times more active than CPM B. CPM B was slightly more active than CFX and APC. Both CPMs were also effective against APC-resistant strains.

Against *Enterobacter* and *Citrobacter*, which were insensitive or resistant to APC or CFX, CPMs A and B showed sufficient activity.

At a concentration of 12.5 μg/ml or less, CPM A inhibited 90% of the strains of *Serratia*, most of which were resistant to CFX and APC. CPM B was eight times less active than CPM A.

The activity of CPM A against indole-positive *Proteus* was the greatest among four antibiotics tested, and all strains were inhibited at a concentration of 3.13 μg or less of CPM A per ml. No resistant strains to CPM B were detected; CPM B was about four times less active than CFX. Against indole-negative *Proteus*, CPM A was

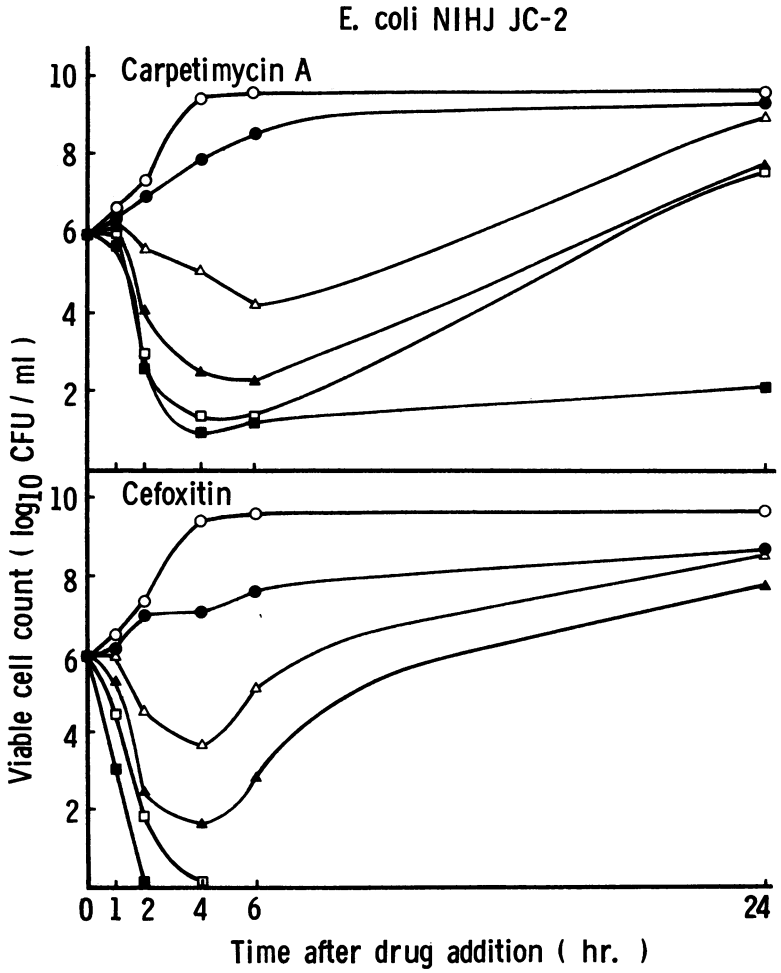


Fig. 2 continued.

TABLE 6. β-Lactamase inhibitory activity of CPMs A and B and CVA

β-Lactamase source (type)[a]	Substrate[b]	Sp act[c] (U/mg of protein)	50% Inhibitory concn (ng/ml)[d]:		
			CPM A	CPM B	CVA[e]
C. freundii 24 (CSase)	CER	93.3	28	31	>10,000
P. morganii 41 (CSase)	CER	21.4	79	290	>10,000
P. aeruginosa 48 (CSase)	CER	34.7	1,000	2,000	>10,000
P. vulgaris 69 (CXase)	CER	83.3	0.52	0.74	13.1
E. coli ML1410 REC1[f] (PCase I)	PCG	41.4	1.61	0.28	9.2
E. coli 59[f] (PCase II)	PCG	1.13	7.1	4.0	150
E. coli 68[f] (PCase IV)	PCG	8.71	48	26	8.2

[a] Classification of Mitsuhashi and Inoue (10, 11): CSase, cephalosporinase; CXase, cefuroximase; PCase, penicillinase. PCase I, II, and IV are identical to TEM-type, oxacillin-hydrolyzing, and carbenicillin-hydrolyzing β-lactamase, respectively.

[b] CER, Cephaloridine.

[c] The hydrolysis of PCG was assayed by a modification of the Novick microiodometric method, and the hydrolysis of CER was measured by the direct spectrophotometric method.

[d] Before the addition of substrate (100 μM, final concentration), the inhibitor was incubated with enzyme (~ 0.05 U) at 30°C for 5 min.

[e] CVA was preincubated for 15 min.

[f] PCase was plasmid mediated.

the most active, and CPM B was less active than APC, CFX, and CEZ.

Activity against APC-resistant bacteria. The activities of CPMs A and B against various species of APC-resistant bacteria are shown in Table 4. Most of the APC-resistant strains were susceptible to CPMs A and B. CPMs A and B inhibited CFX-resistant bacteria, but CPM A was 4 to 32 times more active than CPM B.

Effect of inoculum size, medium pH, and horse serum. To test the effect of several factors on the activity of CPM A, the MICs and MBCs of CPM A against *S. aureus* and *E. coli* were determined under various conditions (Table 5). Changes in inoculum size from 10^4 to 10^8 CFU per ml of *S. aureus* caused no significant effect.

However, an increase of the inoculum size of *E. coli* resulted in a slight increase in the MICs and MBCs of CPM A. The activity of CPM A against both bacteria reduced in the medium of alkaline pH and tended to be more affected than that of CFX.

The MICs of CPM A against *S. aureus* were not affected by the addition of 10 to 50% horse serum to the medium, whereas the MBCs were significantly reduced. Against *E. coli*, the MICs and MBCs of CPM A fluctuated somewhat after the addition of horse serum.

Bactericidal activity. Figure 2 shows the bactericidal activity of CPM A compared with that of CFX. Against *S. aureus*, a significant bactericidal activity was observed at the concentration of 0.39 μg of CPM A per ml (1 MIC) and 1.56 μg of CFX per ml (1 MIC). Against *E. coli*, an apparent decrease in living bacterial cell count was seen with 0.05 μg of CPM A per ml (1 MIC) and 6.25 μg of CFX per ml (1 MIC).

β-Lactamase inhibitory activity. Concentrations giving 50% inhibition of CPMs A and B against various types of β-lactamase (10, 11) in comparison with those of CVA were determined (Table 6).

CPMs A and B inhibited widely different types of enzymes at low concentrations, except for one strain of *P. aeruginosa*. The enzymes that were especially well inhibited by CPMs A and B were CXase (*P. vulgaris* 69), a TEM-type (*E. coli* ML1410 REC1), and an oxacillinase type (*E. coli* 59). Furthermore, CPMs A and B inhibited CSase's, which were known to be insensitive to CVA.

The inhibitory activity of CPM B against PCase was slightly more active than that of CPM A, whereas CPM B against CSase and CXase was somewhat less active than CPM A.

The K_m and K_i values were determined from the kinetic studies of β-lactamases for PCG, CPMs A and B, and CVA (Table 7). The affinities of CPMs A and B to three types of PCase were found to be higher than the affinity of CVA, which suggest that CPMs A and B are potent β-lactamase inhibitors.

Synergistic activity. The combined effects of CPMs A and B with the β-lactam antibiotics commonly used are shown in Table 8.

Combined with CBC, APC, or CEZ, CPMs A and B showed synergistic activity against β-lactamase-producing bacteria.

DISCUSSION

CPMs A and B exhibited broad-spectrum activities against gram-positive and gram-negative aerobic and anaerobic bacteria, including β-lactamase-producing strains. CPM A showed potent antimicrobial activity against various species of clinical isolates and inhibited more than

TABLE 7. Kinetics of β-lactamase inhibition by CPMs A and B and CVA

β-Lactamase source (type)[a]	K_m (μM)[b]	K_i (μM)[b]		
		CPM A	CPM B	CVA
E. coli ML1410 REC1 (PCase I)	31	0.0052	0.0050	0.20
E. coli 59 (PCase II)	8.2	0.88	0.94	13
E. coli 68 (PCase IV)	20	0.51	0.25	1.6

[a] See Table 6, footnote *a*.
[b] The K_m and K_i values were determined with PCG as a substrate.

TABLE 8. Synergistic activity of CPMs A and B with selected β-lactam antibiotics on agar plates

Organism	Antibiotic concn in agar plate (μg/ml)		Inhibition zone (mm)[a] of:	
			CPM A	CPM B
E. coli ML1410 REC1	APC	0	19.4	11.1
		100	28.2	34.4
K. pneumoniae 47	APC	0	15.4	Trace
		100	26.4	25.7
P. aeruginosa 9	CBC	0	—[b]	—
		400	13.8	10.6
C. freundii 24	APC	0	17.6	Trace
		100	21.8	10.6
P. vulgaris 69	CEZ	0	12.8	—
		100	21.7	15.4

[a] Disks (8 mm in diameter) containing 25 μl of CPM A or B solution (0.1 mg/ml) were placed on the agar plate with APC, CBC, or CEZ and incubated for 18 h at 37°C.
[b] —, No inhibition zone.

90% of clinical isolates of *Staphylococcus, E. coli, Klebsiella, Enterobacter, Citrobacter*, and indole-positive and -negative *Proteus* at a concentration of 3.13 μg/ml. However, CPM A showed relatively weak activity against *Serratia* and poor activity against *P. aeruginosa*.

CPM B, a sulfuric acid ester of CPM A, exhibited an antimicrobial spectrum similar to that of CPM A, but its activity was 8 to 64 times less than that of CPM A.

On CPMs, therefore, sulfoxy type at the position C-8 was less active than hydroxy type. On olivanic acid derivatives, however, sulfoxy types at the position C-8 were slightly more effective than hydroxy types (2).

The difference between our data on CPMs and the results on olivanic acid derivatives shows the complication of structure-activity relationships among carbapenem antibiotics. Furthermore, CPMs A and B possessed the side chain with sulfoxide at the position C-3, as do MM4550 (2) and asparenomycin A. The activity of CPM A seems to be the highest among these sulfoxy-type carbapenem antibiotics.

PS-5 (16), olivanic acid derivatives (8), thienamycin (H. Kroop, J. S. Kahan, F. M. Kahan, J. Sundelof, G. Darland, and J. Birnbaum, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother, 16th, Chicago, Ill., abstr. no. 228, 1976), and CVA (18) have been shown to possess β-lactamase inhibitory activities. CPMs A and B also showed inhibitory activity against various types of β-lactamases. But these activities differed from each other, depending on the enzymes. Similar results have been reported by Okonogi et al. (17). They revealed that the inhibition of β-lactamase by C-19393 S_2 (CPM B) and H_2 (CPM A) was of a progressive type except for the inhibition of TEM-type enzyme by C-19393 H_2. The K_i values of CPMs A and B for three types of PCase were similar to each other and lower than the K_i values of *N*-formimidoyl thienamycin (MK0787) reported by Toda et al. (21), which suggests that CPMs A and B are more effective inhibitors for PCase than is *N*-formimidoyl thienamycin. It is interesting that CPMs A and B exhibit similar affinities to β-lactamase, despite the relatively weak antimicrobial activity of CPM B.

Recently, Nozaki et al. (14) reported that C-19393 S_2 and C-19393 H_2 showed the highest affinity for penicillin-binding protein 2, and the binding affinity of C-19393 H_2 to penicillin-binding proteins was remarkably higher than that of C-19393 S_2. They further showed that the inhibitory activity of C-19393 H_2 in peptidoglycan synthesis was also more potent than that of C-19393 S_2. Therefore, the difference of antimicrobial activity between CPMs A and B may be due biochemically to the difference of binding affinity to penicillin-binding proteins and of inhibitory activity in peptidoglycan synthesis.

However, the difference of the permeability of CPMs A and B to target sites in bacterial cells remains unsolvable as a considerable biochemical mechanism.

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