

In Vitro and In Vivo Antibacterial Activities of CS-023 (RO4908463), a Novel Parenteral Carbapenem

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Received 1 February 2005/Returned for modification 28 March 2005/Accepted 15 May 2005

CS-023 (RO4908463, formerly R-115685) is a novel 1 β -methylcarbapenem with 5-substituted pyrrolidin-3-ylthio groups, including an amidine moiety at the C-2 position. Its antibacterial activity was tested against 1,214 clinical isolates of 32 species and was compared with those of imipenem, meropenem, ceftazidime, ceftriaxone, ampicillin, amikacin, and levofloxacin. CS-023 exhibited a broad spectrum of activity against gram-positive and -negative aerobes and anaerobes, including methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis*, penicillin-resistant *Streptococcus pneumoniae* (PRSP), β -lactamase-negative ampicillin-resistant *Haemophilus influenzae*, and *Pseudomonas aeruginosa*. CS-023 showed the most potent activity among the compounds tested against *P. aeruginosa* and MRSA, with MICs at which 90% of isolates tested were inhibited of 4 μ g/ml and 8 μ g/ml, respectively. CS-023 was stable against hydrolysis by the β -lactamases from *Enterobacter cloacae* and *Proteus vulgaris*. CS-023 also showed potent activity against extended-spectrum β -lactamase-producing *Escherichia coli*. The in vivo efficacy of CS-023 was evaluated with a murine systemic infection model induced by 13 strains of gram-positive and -negative pathogens and a lung infection model induced by 2 strains of PRSP (serotypes 6 and 19). Against the systemic infections with PRSP, MRSA, and *P. aeruginosa* and the lung infections, the efficacy of CS-023 was comparable to those of imipenem/cilastatin and vancomycin (tested against lung infections only) and superior to those of meropenem, ceftriaxone, and ceftazidime (tested against *P. aeruginosa* infections only). These results suggest that CS-023 has potential for the treatment of nosocomial bacterial infections by gram-positive and -negative pathogens, including MRSA and *P. aeruginosa*.

Carbapenems, such as imipenem/cilastatin (3), panipenem/betamipron (7), and meropenem (27), are effective in patients with nosocomial infections, since they have an extremely wide antibacterial spectrum and strong activity against gram-positive and -negative aerobic and anaerobic bacteria, including many resistant pathogens. However, carbapenems do not show high activity against methicillin-resistant *Staphylococcus aureus* (MRSA), and the emergence of carbapenem-resistant pathogens has reduced the clinical efficacy of carbapenems (9). MRSA and *Pseudomonas aeruginosa* are major pathogens for patients who have nosocomial infections (11). Therefore, there is an urgent need for new antibacterial agents effective against such highly resistant pathogens.

In the process of research for new carbapenem compounds with a broad spectrum, potent activity against MRSA as well as *P. aeruginosa*, and a longer half-life, we synthesized a novel 1 β -methyl carbapenem, CS-023 (RO4908463, formerly R-115685), (-)-(4*R*,5*S*,6*S*)-3-[[[(3*S*,5*S*)-5-[(*S*)-3-(2-guanidinoacetyl)amino]pyrrolidin-1-ylcarbonyl]-1-methylpyrrolidin-3-yl]thio]-6-[(*R*)-1-hydroxyethyl]-4-methyl-7-oxo-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (Fig. 1).

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In this study, we investigated the in vitro and in vivo antibacterial activities of CS-023 in comparison with those of other carbapenems, i.e., imipenem and meropenem, parenteral cepheps, i.e., ceftazidime and ceftriaxone, a penicillin, ampicillin, a quinolone, levofloxacin, and an aminoglycoside, amikacin.

(This work was presented in part at the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Canada, September 2000 [F-1230, F-1231, F-1232].)

MATERIALS AND METHODS

Antibiotics. CS-023 was synthesized at Sankyo Research Laboratories, Tokyo, Japan. Imipenem, meropenem, ceftazidime, and ceftriaxone were obtained from the National Institute of Infectious Diseases, Tokyo, Japan. Levofloxacin was extracted at Sankyo from a commercial formula (Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan). The following compounds were obtained commercially: cefotaxime (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan); ampicillin (Meiji

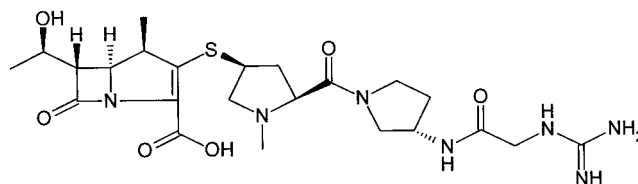


FIG. 1. Chemical structure of CS-023 (RO4908463).

TABLE 1. Activities of CS-023 against Japanese clinical isolates

Organism (no. of strains)	Compound	MIC ($\mu\text{g/ml}$) ^a		
		Range	50%	90%
Methicillin-susceptible ^b <i>S. aureus</i> (48)	CS-023	0.06–0.25	0.12	0.12
	Imipenem	≤ 0.03	≤ 0.03	≤ 0.03
	Meropenem	≤ 0.03 –0.25	0.06	0.12
	Ceftriaxone	1–8	2	4
	Ampicillin	≤ 0.03 –>32	1	>32
	Amikacin	0.25–16	2	8
	Levofloxacin	≤ 0.03 –0.5	0.12	0.12
Methicillin-resistant ^c <i>S. aureus</i> (52)	CS-023	0.12–32	4	8
	Imipenem	≤ 0.03 –>32	32	>32
	Meropenem	0.12–>32	16	32
	Ceftriaxone	4–>32	>32	>32
	Ampicillin	16–>32	32	>32
	Amikacin	0.5–>32	8	32
	Levofloxacin	0.12–>32	4	>32
Methicillin-susceptible ^d <i>S. epidermidis</i> (48)	CS-023	≤ 0.03 –0.06	≤ 0.03	0.06
	Imipenem	≤ 0.03	≤ 0.03	≤ 0.03
	Meropenem	≤ 0.03 –0.12	0.06	0.12
	Ceftriaxone	0.5–4	1	2
	Ampicillin	≤ 0.03 –>32	0.12	8
	Amikacin	0.12–8	0.5	1
	Levofloxacin	0.06–4	0.25	0.5
Methicillin-resistant ^e <i>S. epidermidis</i> (49)	CS-023	≤ 0.03 –8	0.5	4
	Imipenem	≤ 0.03 –32	0.12	8
	Meropenem	0.12–32	1	8
	Ceftriaxone	1–>32	16	>32
	Ampicillin	0.06–>32	8	>32
	Amikacin	0.06–>32	2	16
	Levofloxacin	0.12–>32	2	16
<i>S. haemolyticus</i> (30)	CS-023	≤ 0.03 –4	0.12	1
	Imipenem	≤ 0.03 –32	≤ 0.03	0.25
	Meropenem	≤ 0.03 –16	0.25	4
	Ceftriaxone	0.5–>32	8	32
	Ampicillin	≤ 0.03 –>32	8	32
	Amikacin	0.06–8	0.25	2
	Levofloxacin	0.06–32	0.12	8
<i>S. pyogenes</i> (25)	CS-023	≤ 0.03	≤ 0.03	≤ 0.03
	Imipenem	≤ 0.03	≤ 0.03	≤ 0.03
	Meropenem	≤ 0.03	≤ 0.03	≤ 0.03
	Ceftriaxone	≤ 0.03	≤ 0.03	≤ 0.03
	Ampicillin	≤ 0.03	≤ 0.03	≤ 0.03
	Amikacin	32–>32	>32	>32
	Levofloxacin	0.25–8	0.5	2
<i>S. agalactiae</i> (25)	CS-023	≤ 0.03 –0.06	≤ 0.03	0.06
	Imipenem	≤ 0.03	≤ 0.03	≤ 0.03
	Meropenem	≤ 0.03 –0.06	0.06	0.06
	Ceftriaxone	≤ 0.03 –0.12	0.06	0.06
	Ampicillin	0.06–0.12	0.12	0.12
	Amikacin	>32	>32	>32
	Levofloxacin	0.5–1	1	1
<i>S. oralis</i> (25)	CS-023	≤ 0.03 –4	≤ 0.03	2
	Imipenem	≤ 0.03 –2	≤ 0.03	2
	Meropenem	≤ 0.03 –4	≤ 0.03	2
	Ceftriaxone	≤ 0.03 –>32	0.12	4
	Ampicillin	≤ 0.03 –32	0.12	8
	Amikacin	16–>32	>32	>32
	Levofloxacin	1–2	1	2
<i>S. mitis</i> (24)	CS-023	≤ 0.03 –4	≤ 0.03	1
	Imipenem	≤ 0.03 –2	≤ 0.03	1

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TABLE 1—Continued

Organism (no. of strains)	Compound	MIC ($\mu\text{g/ml}$) ^a		
		Range	50%	90%
	Meropenem	≤ 0.03 –4	≤ 0.03	1
	Ceftriaxone	≤ 0.03 –4	0.06	4
	Ampicillin	≤ 0.03 –16	0.12	16
	Amikacin	8–>32	32	>32
	Levofloxacin	0.5–32	1	32
<i>S. sanguis</i> (19)	CS-023	≤ 0.03 –0.25	≤ 0.03	0.25
	Imipenem	≤ 0.03 –0.12	≤ 0.03	0.06
	Meropenem	≤ 0.03 –0.5	≤ 0.03	0.25
	Ceftriaxone	≤ 0.03 –0.5	0.12	0.5
	Ampicillin	≤ 0.03 –2	0.06	1
	Amikacin	16–>32	>32	>32
	Levofloxacin	0.5–8	1	2
Penicillin-susceptible ^f <i>S. pneumoniae</i> (60)	CS-023	≤ 0.03	≤ 0.03	≤ 0.03
	Imipenem	≤ 0.03	≤ 0.03	≤ 0.03
	Meropenem	≤ 0.03	≤ 0.03	≤ 0.03
	Ceftriaxone	≤ 0.03 –0.5	0.12	0.5
	Ampicillin	≤ 0.03 –0.06	≤ 0.03	≤ 0.03
	Amikacin	8–>32	>32	>32
	Levofloxacin	0.25–1	0.5	0.5
Penicillin-intermediate ^g <i>S. pneumoniae</i> (42)	CS-023	≤ 0.03 –0.25	0.06	0.12
	Imipenem	≤ 0.03 –0.25	0.06	0.12
	Meropenem	≤ 0.03 –0.5	0.12	0.25
	Ceftriaxone	≤ 0.03 –0.5	0.5	0.5
	Ampicillin	≤ 0.03 –2	0.5	2
	Amikacin	16–>32	32	>32
	Levofloxacin	0.25–1	0.5	0.5
Penicillin-resistant ^h <i>S. pneumoniae</i> (47)	CS-023	0.06–0.5	0.12	0.25
	Imipenem	0.06–0.5	0.25	0.5
	Meropenem	0.25–0.5	0.5	0.5
	Ceftriaxone	0.5–4	0.5	0.5
	Ampicillin	0.5–8	2	4
	Amikacin	32–>32	>32	>32
	Levofloxacin	0.5–2	0.5	1
<i>E. faecalis</i> (25)	CS-023	0.25–8	2	8
	Imipenem	0.5–2	1	2
	Meropenem	2–16	4	16
	Ceftriaxone	2–>32	>32	>32
	Ampicillin	0.5–>32	1	2
	Amikacin	32–>32	>32	>32
	Levofloxacin	0.5–>32	2	>32
<i>E. faecium</i> (24)	CS-023	2–>32	>32	>32
	Imipenem	0.5–>32	>32	>32
	Meropenem	8–>32	>32	>32
	Ceftriaxone	>32	>32	>32
	Ampicillin	2–>32	>32	>32
	Amikacin	32–>32	>32	>32
	Levofloxacin	2–>32	>32	>32
<i>P. asaccharolyticus</i> (21)	CS-023	≤ 0.03	≤ 0.03	≤ 0.03
	Imipenem	≤ 0.03	≤ 0.03	≤ 0.03
	Meropenem	≤ 0.03	≤ 0.03	≤ 0.03
	Ceftriaxone	≤ 0.03 –1	≤ 0.03	0.5
	Levofloxacin	0.5–>32	2	32
<i>P. anaerobius</i> (25)	CS-023	≤ 0.03 –4	0.12	2
	Imipenem	≤ 0.03 –1	≤ 0.03	0.5
	Meropenem	≤ 0.03 –2	0.12	2
	Ceftriaxone	0.06–8	0.25	4
	Levofloxacin	0.12–32	0.25	32

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TABLE 1—Continued

Organism (no. of strains)	Compound	MIC ($\mu\text{g/ml}$) ^a		
		Range	50%	90%
<i>P. magnus</i> (25)	CS-023	≤ 0.03 –0.06	≤ 0.03	≤ 0.03
	Imipenem	≤ 0.03 –0.06	≤ 0.03	≤ 0.03
	Meropenem	≤ 0.03 –0.12	≤ 0.03	≤ 0.03
	Ceftriaxone	≤ 0.03 –8	2	8
	Levofloxacin	0.06–>32	2	>32
<i>P. acnes</i> (25)	CS-023	≤ 0.03 –0.12	≤ 0.03	0.12
	Imipenem	≤ 0.03	≤ 0.03	≤ 0.03
	Meropenem	≤ 0.03 –0.5	≤ 0.03	0.5
	Ceftriaxone	≤ 0.03 –0.5	0.12	0.5
	Levofloxacin	0.12–0.5	0.25	0.5
<i>M. catarrhalis</i> (25)	CS-023	≤ 0.03	≤ 0.03	≤ 0.03
	Imipenem	≤ 0.03 –0.12	0.06	0.06
	Meropenem	≤ 0.03	≤ 0.03	≤ 0.03
	Ceftazidime	0.06–0.25	0.06	0.12
	Ceftriaxone	0.06–2	0.5	1
	Ampicillin	0.5–32	8	16
	Amikacin	0.5–2	0.5	1
	Levofloxacin	≤ 0.03	≤ 0.03	≤ 0.03
Ampicillin-susceptible ⁱ <i>H. influenzae</i> (65)	CS-023	≤ 0.03 –0.5	≤ 0.03	0.25
	Imipenem	0.12–4	0.5	0.5
	Meropenem	≤ 0.03 –0.5	≤ 0.03	0.25
	Ceftazidime	≤ 0.03 –8	0.06	0.5
	Ceftriaxone	≤ 0.03 –0.5	≤ 0.03	0.25
	Ampicillin	0.12–2	0.5	2
	Amikacin	2–16	8	16
	Levofloxacin	≤ 0.03 –0.5	≤ 0.03	≤ 0.03
β -lactamase-negative ampicillin-resistant ^f <i>H. influenzae</i> (11)	CS-023	≤ 0.03 –0.5	0.12	0.25
	Imipenem	0.25–4	0.5	4
	Meropenem	≤ 0.03 –0.5	0.25	0.25
	Ceftazidime	0.12–0.5	0.25	0.25
	Ceftriaxone	0.12–0.25	0.12	0.25
	Ampicillin	4	4	4
	Amikacin	2–8	4	8
	Levofloxacin	≤ 0.03	≤ 0.03	≤ 0.03
<i>E. coli</i> (25)	CS-023	≤ 0.03 –0.12	≤ 0.03	≤ 0.03
	Imipenem	0.06–0.5	0.12	0.25
	Meropenem	≤ 0.03 –0.25	≤ 0.03	≤ 0.03
	Ceftazidime	0.06–2	0.25	1
	Ceftriaxone	≤ 0.03 –2	≤ 0.03	0.25
	Ampicillin	2–>32	4	>32
	Amikacin	1–8	2	4
	Levofloxacin	≤ 0.03 –>32	0.12	2
<i>C. freundii</i> (25)	CS-023	≤ 0.03 –0.06	≤ 0.03	0.06
	Imipenem	0.06–1	0.12	1
	Meropenem	≤ 0.03 –0.06	≤ 0.03	0.06
	Ceftazidime	0.06–>32	0.25	>32
	Ceftriaxone	≤ 0.03 –>32	0.12	>32
	Ampicillin	16–>32	>32	>32
	Amikacin	0.5–2	1	2
	Levofloxacin	≤ 0.03 –1	0.06	0.5
<i>K. pneumoniae</i> (25)	CS-023	≤ 0.03 –0.12	≤ 0.03	0.06
	Imipenem	0.06–1	0.12	0.5
	Meropenem	≤ 0.03 –0.06	≤ 0.03	≤ 0.03
	Ceftazidime	0.06–16	0.25	2
	Ceftriaxone	≤ 0.03 –0.5	0.06	0.25
	Ampicillin	16–>32	>32	>32
	Amikacin	0.5–2	1	2
	Levofloxacin	≤ 0.03 –8	0.06	1

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TABLE 1—Continued

Organism (no. of strains)	Compound	MIC ($\mu\text{g/ml}$) ^a		
		Range	50%	90%
<i>K. oxytoca</i> (25)	CS-023	≤ 0.03 –0.12	≤ 0.03	0.12
	Imipenem	0.12–0.5	0.25	0.5
	Meropenem	≤ 0.03 –0.12	≤ 0.03	0.12
	Ceftazidime	0.06–4	0.25	2
	Ceftriaxone	≤ 0.03 –>32	0.06	>32
	Ampicillin	32–>32	>32	>32
	Amikacin	0.5–4	1	2
	Levofloxacin	≤ 0.03 –16	≤ 0.03	8
<i>E. cloacae</i> (25)	CS-023	≤ 0.03 –2	0.06	1
	Imipenem	0.25–4	0.5	1
	Meropenem	≤ 0.03 –4	0.12	0.5
	Ceftazidime	0.12–>32	32	>32
	Ceftriaxone	0.12–>32	>32	>32
	Ampicillin	>32	>32	>32
	Amikacin	0.5–32	1	16
	Levofloxacin	≤ 0.03 –16	0.5	4
<i>E. aerogenes</i> (25)	CS-023	≤ 0.03 –0.12	≤ 0.03	0.06
	Imipenem	0.5–2	1	2
	Meropenem	≤ 0.03 –0.12	≤ 0.03	0.06
	Ceftazidime	0.06–>32	0.25	32
	Ceftriaxone	≤ 0.03 –32	0.12	16
	Ampicillin	>32	>32	>32
	Amikacin	0.5–2	2	2
	Levofloxacin	≤ 0.03 –0.12	≤ 0.03	0.06
<i>S. marcescens</i> (50)	CS-023	≤ 0.03 –>32	0.06	0.25
	Imipenem	0.25–>32	2	4
	Meropenem	≤ 0.03 –>32	0.06	0.25
	Ceftazidime	≤ 0.03 –>32	0.12	16
	Ceftriaxone	≤ 0.03 –>32	0.25	>32
	Ampicillin	4–>32	>32	>32
	Amikacin	1–>32	2	8
	Levofloxacin	≤ 0.03 –>32	0.12	2
<i>P. vulgaris</i> (25)	CS-023	0.12–0.25	0.25	0.25
	Imipenem	4–8	4	8
	Meropenem	0.06–0.25	0.12	0.12
	Ceftazidime	0.12–16	0.5	4
	Ceftriaxone	4–>32	>32	>32
	Ampicillin	>32	>32	>32
	Amikacin	1–16	2	4
	Levofloxacin	≤ 0.03 –32	≤ 0.03	0.12
<i>P. mirabilis</i> (25)	CS-023	≤ 0.03 –0.5	0.12	0.25
	Imipenem	0.12–8	4	4
	Meropenem	≤ 0.03 –0.5	0.12	0.12
	Ceftazidime	0.06–>32	0.12	2
	Ceftriaxone	≤ 0.03 –8	≤ 0.03	0.12
	Ampicillin	0.5–>32	2	>32
	Amikacin	1–16	4	4
	Levofloxacin	≤ 0.03 –1	0.06	0.12
<i>M. morgani</i> (25)	CS-023	≤ 0.03 –0.5	0.25	0.25
	Imipenem	4–8	4	4
	Meropenem	0.06–0.25	0.25	0.25
	Ceftazidime	0.06–>32	4	>32
	Ceftriaxone	≤ 0.03 –>32	0.25	32
	Ampicillin	>32	>32	>32
	Amikacin	1–8	2	4
	Levofloxacin	≤ 0.03 –8	≤ 0.03	2
<i>P. rettgeri</i> (25)	CS-023	0.12–4	0.25	4
	Imipenem	0.5–32	2	4
	Meropenem	≤ 0.03 –4	0.12	0.25

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TABLE 1—Continued

Organism (no. of strains)	Compound	MIC ($\mu\text{g/ml}$) ^a		
		Range	50%	90%
	Ceftazidime	0.06->32	>32	>32
	Ceftriaxone	≤ 0.03 ->32	0.25	16
	Ampicillin	1->32	>32	>32
	Amikacin	0.25-32	2	16
	Levofloxacin	≤ 0.03 ->32	0.12	16
<i>P. aeruginosa</i> (total, 100)	CS-023	0.06-32	0.5	4
	Imipenem	0.25-32	1	16
	Meropenem	0.06->32	0.5	16
	Ceftazidime	1->32	4	32
	Ceftriaxone	4->32	>32	>32
	Ampicillin	32->32	>32	>32
	Amikacin	0.5->32	4	16
	Levofloxacin	0.12->32	0.5	16
<i>P. aeruginosa</i> (MIC of IPM, ≤ 8) (79)	CS-023	0.06-16	0.25	1
	Imipenem	0.25-8	1	4
	Meropenem	0.06-16	0.5	2
	Ceftazidime	1->32	2	16
	Ceftriaxone	4->32	>32	>32
	Ampicillin	32->32	>32	>32
	Amikacin	0.5->32	4	16
	Levofloxacin	0.12->32	0.5	4
<i>P. aeruginosa</i> (MIC of IPM, >8) (21)	CS-023	1-32	4	16
	Imipenem	16-32	16	32
	Meropenem	1->32	8	32
	Ceftazidime	2->32	32	>32
	Ceftriaxone	16->32	>32	>32
	Ampicillin	>32	>32	>32
	Amikacin	0.5->32	4	16
	Levofloxacin	0.25->32	1	32
<i>B. cepacia</i> (25)	CS-023	4-32	32	32
	Imipenem	1-32	8	16
	Meropenem	0.25-8	2	4
	Ceftazidime	2-8	4	8
	Ceftriaxone	8->32	>32	>32
	Ampicillin	4->32	>32	>32
	Amikacin	16->32	>32	>32
	Levofloxacin	1-32	4	8
<i>S. maltophilia</i> (25)	CS-023	0.5->32	>32	>32
	Imipenem	32->32	>32	>32
	Meropenem	0.12->32	>32	>32
	Ceftazidime	2->32	32	>32
	Ceftriaxone	>32	>32	>32
	Ampicillin	>32	>32	>32
	Amikacin	>32	>32	>32
	Levofloxacin	0.5-32	1	4
<i>B. fragilis</i> (24)	CS-023	≤ 0.03 -16	0.12	0.5
	Imipenem	≤ 0.03 -4	0.12	0.25
	Meropenem	≤ 0.03 -16	0.12	0.25
	Ceftazidime	8->32	16	>32
	Ceftriaxone	2->32	32	>32
	Levofloxacin	1-32	2	8

^a 50% and 90%, MICs at which 50% and 90%, respectively, of the isolates are inhibited.

^b MIC of oxacillin: 2 $\mu\text{g/ml}$ or lower.

^c MIC of oxacillin: 4 $\mu\text{g/ml}$ or higher.

^d MIC of oxacillin: 0.25 $\mu\text{g/ml}$ or lower.

^e MIC of oxacillin: 0.5 $\mu\text{g/ml}$ or higher.

^f MIC of benzylpenicillin: 0.06 $\mu\text{g/ml}$ or lower.

^g MIC of benzylpenicillin: 0.12 to 1 $\mu\text{g/ml}$.

^h MIC of benzylpenicillin: 2 $\mu\text{g/ml}$ or higher.

ⁱ MIC of ampicillin: 2 $\mu\text{g/ml}$ or lower.

^j MIC of ampicillin: 4 $\mu\text{g/ml}$ or higher.

TABLE 4. Protective effect of CS-023 on systemic infections in mice

Organism (MIC of drug), Challenge dose (CFU/mouse)	Compound	MIC ($\mu\text{g/ml}$)	ED ₅₀ ^a (mg/kg/dose)	95% confidence interval (mg/kg/dose)
Methicillin-susceptible <i>S. aureus</i> Smith ^b (MIC of MIPIC, ^d 0.25 $\mu\text{g/ml}$), 1.3×10^7	CS-023	0.12	0.97	0.61–1.5
	Imipenem/cilastatin	0.016	0.20	NC ^c
	Meropenem	0.06	5.8	3.9–9.9
	Ceftriaxone	4	6.6	NC
Methicillin-susceptible <i>S. aureus</i> 560 ^b (MIC of MIPIC, 1 $\mu\text{g/ml}$), 1.3×10^8	CS-023	0.25	2.6	1.6–4.3
	Imipenem/cilastatin	0.016	0.61	0.40–0.99
	Meropenem	0.12	39	27–58
	Ceftriaxone	4	8.8	NC
Methicillin-resistant <i>S. aureus</i> 507 ^b (MIC of MIPIC, 32 $\mu\text{g/ml}$), 1.6×10^7	CS-023	1	7.9	3.9–12
	Imipenem/cilastatin	0.12	13	8.6–20
	Meropenem	2	>100	NC
	Ceftriaxone	32	39	25–70
Methicillin-susceptible <i>S. epidermidis</i> 1392, ^b 4.8×10^8	CS-023	0.06	0.76	0.47–1.1
	Imipenem/cilastatin	0.016	0.16	0.10–0.24
	Meropenem	0.06	5.8	3.5–9.7
	Ceftriaxone	2	11	NC
Penicillin-susceptible <i>S. pneumoniae</i> 2132 ^b (MIC of PCG, ^e 0.016 $\mu\text{g/ml}$), 1.4×10^2	CS-023	≤ 0.008	0.10	0.071–0.16
	Imipenem/cilastatin	≤ 0.008	0.042	0.010–0.075
	Meropenem	0.016	1.1	0.69–2.0
	Ceftriaxone	0.016	0.13	0.083–0.19
	Ampicillin	0.03	0.48	0.30–0.74
Penicillin-resistant <i>S. pneumoniae</i> 9605 ^b (MIC of PCG, 4 $\mu\text{g/ml}$), 6.9×10^2	CS-023	0.25	0.55	0.38–0.79
	Imipenem/cilastatin	0.25	0.33	0.13–0.52
	Meropenem	0.5	3.9	2.0–6.4
	Ceftriaxone	1	2.1	1.2–4.9
	Ampicillin	4	7.9	4.9–14
<i>E. coli</i> 704, 5.9×10^7	CS-023	≤ 0.008	1.2	0.82–2.5
	Imipenem/cilastatin	0.12	4.9	3.3–10
	Meropenem	0.016	5.9	NC
	Ceftazidime	0.12	3.2	NC
	Ceftriaxone	0.03	0.33	0.19–0.63
<i>C. freundii</i> 1231, ^b 3.7×10^7	CS-023	0.016	0.31	0.17–0.54
	Imipenem/cilastatin	1	1.2	0.81–1.8
	Meropenem	0.016	1.2	0.41–2.0
	Ceftazidime	0.5	3.2	1.7–5.4
	Ceftriaxone	0.12	0.27	0.15–0.55
<i>K. pneumoniae</i> 866, ^b 6.4×10^6	CS-023	0.016	0.67	0.46–1.0
	Imipenem/cilastatin	0.25	1.8	1.2–2.7
	Meropenem	0.03	1.8	1.1–3.0
	Ceftazidime	0.25	0.46	0.29–0.79
	Ceftriaxone	0.25	0.11	NC
<i>E. aerogenes</i> 100, ^b 4.6×10^7	CS-023	0.06	6.5	3.2–15
	Imipenem/cilastatin	2	3.1	1.7–5.0
	Meropenem	0.06	31	NC
	Ceftazidime	0.12	3.1	1.5–5.0
	Ceftriaxone	0.06	2.5	NC
<i>S. marcescens</i> 1850, 6.9×10^7	CS-023	0.016	1.1	0.61–6.7
	Imipenem/cilastatin	0.12	9.4	4.8–74
	Meropenem	0.016	2.5	1.6–5.0
	Ceftazidime	0.12	0.68	0.32–25
	Ceftriaxone	0.25	0.054	0.030–55
<i>P. mirabilis</i> 1262, ^b 4.8×10^8	CS-023	0.25	39	22–260
	Imipenem/cilastatin	4	>100	NC
	Meropenem	0.12	>100	NC
	Ceftazidime	0.25	29	19–43
	Ceftriaxone	0.016	0.28	0.18–0.45

Continued on facing page

TABLE 4—Continued

Organism (MIC of drug), Challenge dose (CFU/mouse)	Compound	MIC ($\mu\text{g/ml}$)	ED ₅₀ ^a (mg/kg/dose)	95% confidence interval (mg/kg/dose)
<i>P. aeruginosa</i> 1008, 2.0×10^7	CS-023	0.12	0.95	0.42–4.8
	Imipenem/cilastatin	1	0.73	0.50–1.1
	Meropenem	0.12	4.2	2.7–12
	Ceftazidime	2	58	38–110
	Ceftriaxone	128	>100	NC

^a Groups of seven mice were challenged with bacterial suspension intraperitoneally, and the compounds were administered by the subcutaneous route immediately and 4 h postinfection.

^b 5% mucin.

^c NC, not calculated.

^d MPIP, oxacillin.

^e PCG, penicillin G.

using *Bacillus subtilis* ATCC 6633 as the bioassay indicator (10). Standard curves were constructed with mouse plasma. For CS-023, assay validation indicated linearity (r^2 value of 0.9959) in the range of 0.625 to 40 $\mu\text{g/ml}$, intra-assay bias ranged from 0.6 to 17.1%, and the coefficient of variation (CV) ranged from 3.7 to 8.6%, indicating acceptable accuracy and precision. The limit of quantitation was 0.625 $\mu\text{g/ml}$ with a bias of 17.1% and a CV of 5.4%. For imipenem, assay validation indicated linearity (r^2 value of 0.9990) in the range of 0.313 to 40 $\mu\text{g/ml}$, intra-assay bias ranged from 1.4 to 13.5%, and the CV ranged from 4.1 to 9.0%, indicating acceptable accuracy and precision. The limit of quantitation was 0.313 $\mu\text{g/ml}$ with a bias of 8.5% and a CV of 4.8%. For meropenem, assay validation indicated linearity (r^2 value of 0.9993) in the range of 0.313 to 40 $\mu\text{g/ml}$, intra-assay bias ranged from 1.8 to 8.3%, and the CV ranged from 2.3 to 11.1%, indicating acceptable accuracy and precision. The limit of quantitation was 0.313 $\mu\text{g/ml}$ with a bias of 1.9% and a CV of 9.2%. For ceftriaxone, assay validation indicated linearity (r^2 value of 0.9974) in the range of 0.625 to 40 $\mu\text{g/ml}$, intra-assay bias ranged from 0.3 to 14.8%, and the CV ranged from 6.8 to 14.4%, indicating acceptable accuracy and precision. The limit of quantitation was 0.625 $\mu\text{g/ml}$ with a bias of 7.1% and a CV of 6.9%. For ceftazidime, assay validation indicated linearity (r^2 value of 0.9925) in the range of 1.25 to 40 $\mu\text{g/ml}$, intra-assay bias ranged from 2.5 to 17.0%, and the CV ranged from 3.8 to 11.7%, indicating acceptable accuracy and precision. The limit of quantitation was 1.25 $\mu\text{g/ml}$ with a bias of 17.0% and a CV of 11.7%. The pharmacokinetic parameters of the concentration in plasma were calculated by one-compartment analysis with the WinNonlin program (Scientific Consulting, Inc.).

Statistical analysis. The statistical significance of the difference between the numbers of viable organisms recovered from the lungs of each treated group and those for the untreated control group was evaluated by a nonparametric Williams' test. A difference between the treated group and the untreated control group was considered to be statistically significant if the P value was <0.05.

RESULTS

Antibacterial activity. The antibacterial activity of CS-023 was determined against 1,214 clinical isolates of 32 species (19 to 149 strains for each species) in comparison with those of imipenem, meropenem, ceftazidime, ceftriaxone, ampicillin, amikacin, and levofloxacin. Table 1 summarizes the MIC ranges and MICs at which 50% and 90% (MIC₉₀) of the clinical isolates were inhibited. CS-023 exhibited a broad antibacterial spectrum against gram-positive and -negative aerobes and anaerobes.

CS-023 showed highly potent activity against methicillin-susceptible *S. aureus* and methicillin-susceptible *S. epidermidis*, with MIC₉₀s of ≤ 0.12 $\mu\text{g/ml}$. Imipenem showed the most potent activity among the reference compounds. Against MRSA, CS-023 was more active than the reference compounds, as indicated by a MIC₉₀ fourfold or more lower than those of the reference compounds (MIC₉₀ for MRSA, 8 $\mu\text{g/ml}$). Against methicillin-resistant *Staphylococcus epidermidis*, the MIC₉₀s of CS-023, imipenem, and meropenem were 4, 8, and 8 $\mu\text{g/ml}$, respectively.

CS-023 showed high activity against most streptococci, including PRSP (MIC₉₀s, ≤ 0.03 to 0.25 $\mu\text{g/ml}$) except for *Streptococcus oralis* and *Streptococcus mitis*. Its activity was comparable to or superior to those of the reference compounds. Although CS-023 exhibited potent activity against *Enterococcus faecalis* (MIC₉₀, 8 $\mu\text{g/ml}$), it was less active than imipenem. All carbapenems were highly active against peptostreptococci.

CS-023 showed high activity against *M. catarrhalis* and ampicillin-susceptible *H. influenzae*, and the activity was comparable to that of meropenem. Against β -lactamase-negative ampicillin-resistant *H. influenzae*, CS-023 was as active as meropenem (MIC₉₀, 0.25 $\mu\text{g/ml}$) and 16-fold more active than imipenem.

CS-023 showed consistently potent activity against the strains of the family *Enterobacteriaceae*, such as *E. coli*, *Klebsiella* spp., and *Proteus* spp., whose activities ranged from ≤ 0.03 to 1 $\mu\text{g/ml}$. The activity of CS-023 against the family *Enterobacteriaceae* except for *Providencia rettgeri* (MIC₉₀, 4 $\mu\text{g/ml}$) was comparable to that of meropenem and stronger than that of imipenem. The activity of CS-023 was stronger than those of cepheims against all members of the family *Enterobacteriaceae* tested, particularly against pathogens that commonly produce β -lactamases (e.g., *Citrobacter freundii*, *Enterobacter* spp., *S. marcescens*, and *Morganella morganii*).

CS-023 showed the most potent activity against *P. aeruginosa* (MIC₉₀, 4 $\mu\text{g/ml}$), being fourfold more active than imipenem, meropenem, and levofloxacin. The MIC₉₀s of CS-023, imipenem, and meropenem against *Burkholderia cepacia* were 32, 16, and 4 $\mu\text{g/ml}$, respectively. None of the carbapenems was highly potent against *Stenotrophomonas maltophilia*, although levofloxacin showed moderate activity. All carbapenems were highly active against *B. fragilis*.

Stability against β -lactamases. Table 2 shows the stability of CS-023, imipenem, meropenem, and ceftriaxone against hydrolysis by β -lactamases from *E. cloacae* and *P. vulgaris*. CS-023, imipenem, and meropenem were quite stable against hydrolysis by both β -lactamases. Ceftriaxone was hydrolyzed by β -lactamase from *P. vulgaris*.

Antibacterial activity of CS-023 against ESBL producers. Table 3 shows the antibacterial activity of CS-023 and reference compounds against the host strain *E. coli* JM109 and its transformants (22), determined by the broth microdilution method. The MICs of CS-023 against transformants containing plasmids carrying TEM- and SHV-type ESBL genes were 0.016 $\mu\text{g/ml}$, showing that the magnitude of increase in MICs

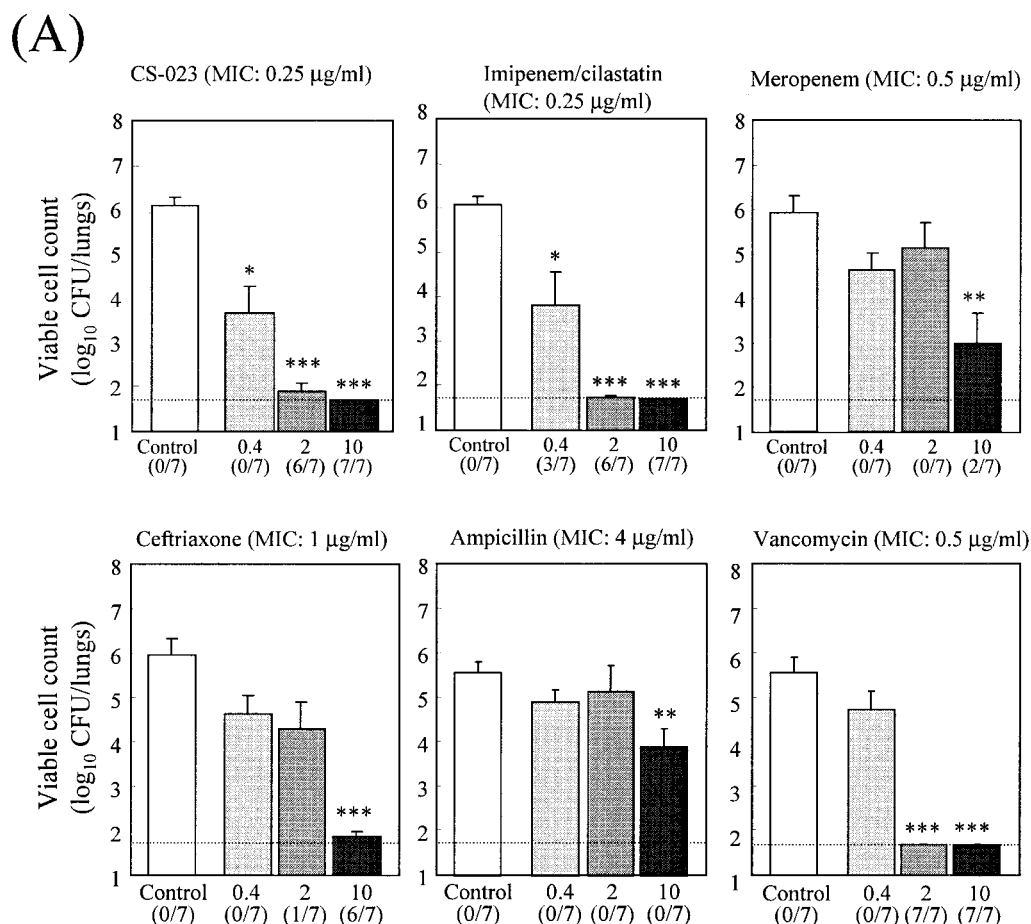


FIG. 2. Therapeutic efficacies of CS-023 and reference compounds against a pneumonia model induced by PRSP 9601 (A) and 10931 (B). Each column and bar represents the mean \pm standard error, respectively ($n = 7$). The indicated doses of each compound were administered subcutaneously twice daily for 2 days beginning 18 h after infection, and the numbers of viable cells in the lungs of mice were determined 66 h after infection. The asterisks indicate a significant difference compared with the results for the control group (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). The dotted line indicates the detection limit. The numbers in parentheses indicate the numbers of culture-negative mice per group whose viable cell counts were below the detection limit.

was two times that with the host. The MIC of CS-023 against the Toho-1 producers increased eight times, compared with that against the host, but was still as low as 0.06 $\mu\text{g/ml}$. Other carbapenems showed similar tendencies. The MICs of cepheps and penicillins against TEM-3, -5, and -9, SHV-type ESBL, and Toho-1 producers increased more than 32 times compared with those against the host strain. The MICs of both amikacin and levofloxacin against all ESBL producers were uniform.

Efficacy against a murine systemic infection model. Table 4, shows the protective effects of CS-023, imipenem/cilastatin, meropenem, ceftriaxone, and ceftazidime on a murine systemic infection model induced by a variety of pathogens. The protective effect of CS-023 was comparable to that of imipenem/cilastatin and stronger than those of the other compounds for infections induced by MRSA 507, PRSP 9605, and *P. aeruginosa* 1008. For the other gram-positive bacterial infections, its protective effect was comparable or inferior to that of imipenem/cilastatin and stronger than those of the other compounds evaluated. For the other gram-negative bacterial infections, its protective effect was generally less active than

that of ceftriaxone and stronger than those of the other compounds evaluated.

Efficacy against a murine pneumonia model induced by PRSP. Fig. 2 shows the therapeutic efficacy of CS-023 and reference compounds with a pneumonia model induced by two PRSP strains. CS-023 exhibited dose-dependent efficacy against both infections at doses of 0.4, 2, and 10 mg/kg. CS-023 showed efficacy comparable with those of imipenem/cilastatin and vancomycin and stronger efficacy than those of the other compounds evaluated against a pneumonia model induced by PRSP 9601 (Fig. 2A). It showed significant reductions in viable cell counts in the lungs at doses of 0.4, 2, and 10 mg/kg. CS-023 showed efficacy comparable to those of imipenem/cilastatin and vancomycin and stronger efficacy than those of the other compounds evaluated against a pneumonia model induced by PRSP 10691 (Fig. 2B). It showed significant reductions in viable cell counts in the lungs at doses of 2 and 10 mg/kg.

Pharmacokinetics of compounds in plasma. Table 5 shows the pharmacokinetic parameters of CS-023 and the reference compounds in plasma after a single subcutaneous administration at 20 mg/kg. The concentrations of CS-023 in plasma were

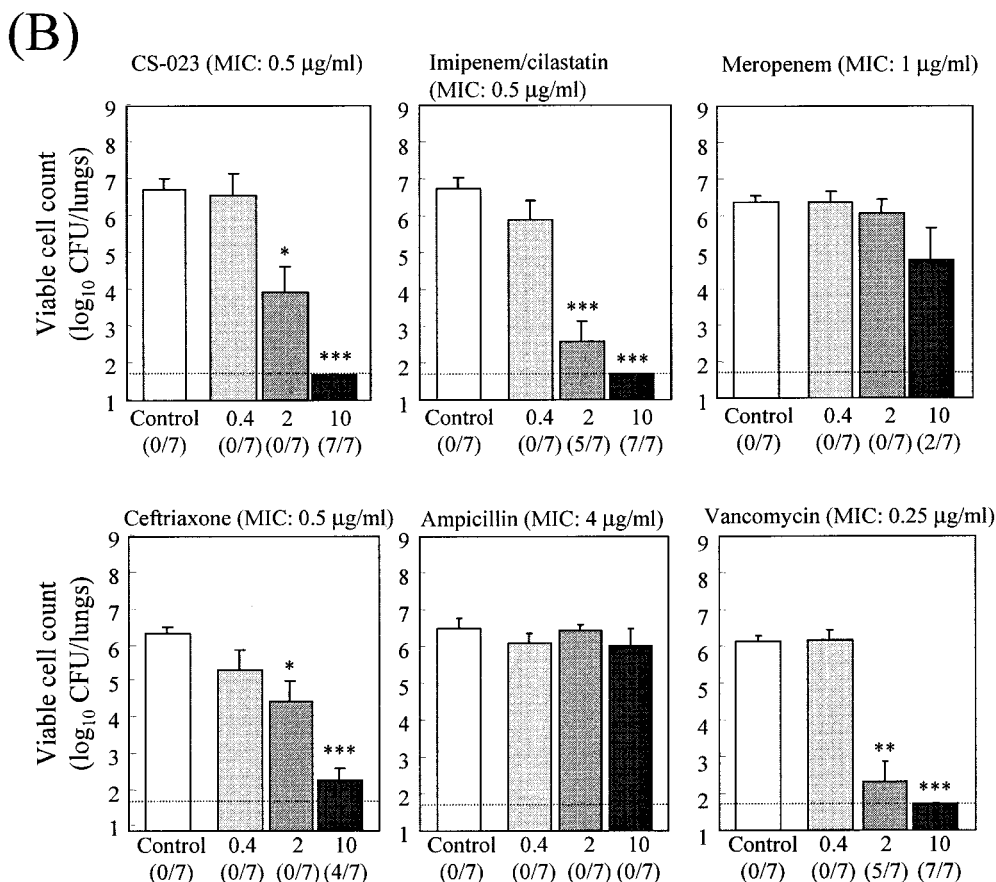


FIG. 2—Continued.

higher than those of meropenem, almost similar to those of imipenem, and lower than those of the other compounds. The mean peak level (\pm standard error) of CS-023 was 20.0 ± 1.9 µg/ml at 0.25 h after administration. At 2 h, the concentration of CS-023 was below the detection limit. The half-life of CS-023 was 0.18 h, compared with 0.14 h for imipenem/cilastatin, 0.10 h for meropenem, 0.21 h for ceftazidime, and 0.83 h for ceftriaxone.

DISCUSSION

CS-023 showed potent antibacterial activity with a broad antibacterial spectrum that encompasses a wide range of gram-positive and -negative aerobic and anaerobic pathogens. Overall, CS-023 demonstrated in vitro activity comparable to that of imipenem against most isolates of gram-positive pathogens and similar to that of meropenem against gram-negative pathogens. These findings were consistent with the results obtained by using United States and European clinical isolates (6, 23). A characteristic of CS-023 is its improved activity against MRSA and *P. aeruginosa* compared with the other carbapenems tested. We have evaluated the stability against commercially available β-lactamases only in this study; however, CS-023 was very stable against two types of β-lactamases of *Enterobacteriaceae*, such as *E. cloacae* and *P. vulgaris*, compared with ceftriaxone. This suggests that the high stability of CS-023 against these β-lactamases is one reason for its potent activity against clinical isolates of the family *Enterobacteri-*

aceae. However, 1 of 50 clinical isolates of *S. marcescens* tested was resistant to carbapenems, including CS-023. Recently, newer β-lactamases hydrolyzing imipenem have been identified from clinical isolates of *S. marcescens* (17). The carbapenem-resistant *S. marcescens* strain used in this study might produce carbapenem-hydrolyzing β-lactamase. The stability of CS-023 against other groups of β-lactamases, such as metallo- and OXA-type β-lactamases, remains to be elucidated.

The MICs of parenteral cepheims and penicillins tested against TEM- and SHV-type ESBL producers were increased more than 32 times over those against the host strain. The antibacterial activities of CS-023 and other carbapenems were virtually unchanged against the ESBL producers tested. This property is one of the advantages of CS-023 as well as other carbapenems over cepheims and penicillins.

TABLE 5. Pharmacokinetics of CS-023 in mouse plasma^a

Compound	T _{max} (h)	C _{max} (µg/ml)	t _{1/2}	AUC ₀₋₂ (µg · h/ml)
CS-023	0.25	20.0 ± 1.9	0.18	12.7
Imipenem/cilastatin	0.25	24.9 ± 1.7	0.14	13.5
Meropenem	0.083	14.6 ± 2.1	0.10	6.70
Ceftazidime	0.25	30.9 ± 3.1	0.21	19.3
Ceftriaxone	0.25	45.4 ± 4.6	0.83	51.2

^a Abbreviations: T_{max}, time to maximum concentration of compound in plasma; C_{max}, maximum concentration of compound in plasma; t_{1/2}, half-life; AUC₀₋₂, area under the concentration-time curve from time zero to 2 h.

The therapeutic efficacy of CS-023 was comparable to that of imipenem/cilastatin in a systemic infection model induced by MRSA, PRSP, and *P. aeruginosa*. CS-023 also showed efficacy comparable to those of imipenem/cilastatin and vancomycin in a pneumonia model induced by two serotypes of PRSP. Plasma concentrations were different among the compounds tested. Since meropenem is unstable against mouse renal dehydropeptidase I (4, 24), the difference could be due to faster elimination than the other compounds evaluated. Ceftriaxone maintained a relatively high concentration and showed a long half-life, this result being consistent with that of a previous report (12). In the present study, the pharmacokinetics of each compound in uninfected mice was determined. The relationship between pharmacokinetic data and therapeutic efficacy was not evaluated, since the underlying infection may have a dramatic effect on systemic pharmacokinetics as well as distribution properties at the infected site depending on the compounds. However, therapeutic efficacy of CS-023 is considered to reflect the in vitro potent antibacterial activity of CS-023. One of the reasons for this would be the low level of protein binding of CS-023 in serum. The protein binding values of CS-023 were 17.6% and 9.5% in mouse and human sera, respectively (T. Shibayama et al., unpublished data). The protein binding values found for CS-023, imipenem (2%) (8), meropenem (33.8%) (21), and ceftazidime (17.9%) (16) have a similar tendency in mice, since protein binding values of less than 70% for β -lactams would have minimal impact on penetration (26). On the contrary, the protein binding of ceftriaxone in mice has been reported to be >80% (12). It is suggested that the effectiveness of CS-023 in mouse models results from its low level of protein binding.

Human plasma pharmacokinetics of CS-023 predicted a longer half-life than that of imipenem/cilastatin by the Detrick plot method (2), which is based on the allometric relationship between body weight and pharmacokinetic parameters, although mouse plasma pharmacokinetics of CS-023 was comparable to that of imipenem/cilastatin (20). This prediction was confirmed by demonstrating that CS-023 (1.5 to 2.1 h) showed a much longer half-life than imipenem/cilastatin (1.0 h) (13) and meropenem (0.9 h) (19) in a study with healthy male volunteers (18). These results suggest that CS-023 could show more-potent efficacy in clinical practice than it showed with the mouse model.

In conclusion, the new parenteral administered carbapenem CS-023 is considered to be an extremely promising compound for further evaluation. Its antibacterial activity would cover not only *P. aeruginosa* but also MRSA encountered in the treatment of nosocomial infections. Clinical studies on CS-023 are in progress globally in Japan, Europe, and the United States.

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