In Vitro Activity of S-3578, a New Broad-Spectrum Cephalosporin Active against Methicillin-Resistant Staphylococci

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Received 5 July 2002/Returned for modification 1 October 2002/Accepted 10 December 2002

The *in vitro* antibacterial activity of S-3578, a new parenteral cephalosporin, against clinical isolates was evaluated. The MICs of the drug at which 90% of the isolates were inhibited were 4 µg/ml for methicillin-resistant *Staphylococcus aureus* (MRSA) and 2 µg/ml for methicillin-resistant *Staphylococcus epidermidis*, which were fourfold higher than and equal to those of vancomycin, respectively. The anti-MRSA activity of S-3578 was considered to be due to its high affinity for penicillin-binding protein 2a (50% inhibitory concentration, 4.5 µg/ml). In time-kill studies with 10 strains each of MRSA and methicillin-susceptible *S. aureus*, S-3578 caused more than a 4-log₁₀ decrease of viable cells on the average at twice the MIC after 24 h of exposure, indicating that it had potent bactericidal activity. Furthermore, in population analysis of MRSA strains with heterogeneous or homogeneous resistance to imipenem, no colonies emerged from about 10⁹ cells on agar plates containing twice the MIC of S-3578, suggesting the low frequency of emergence of S-3578-resistant strains from MRSA. S-3578 was also highly active against penicillin-resistant *Streptococcus pneumoniae* (PRSP), with a MIC₉₀ of 1 µg/ml, which was comparable to that of ceftriaxone. S-3578 also had antibacterial activity against a variety of gram-negative bacteria including *Pseudomonas aeruginosa*, though its activity was not superior to that of cefepime. In conclusion, S-3578 exhibited a broad antibacterial spectrum and, particularly, had excellent activity against gram-positive bacteria including methicillin-resistant staphylococci and PRSP. Thus, S-3578 was considered to be worthy of further evaluation.

Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (MRCNS) cause serious problems in nosocomial infection. The major factor in the mechanism of resistance to β-lactams in MRSA and MRCNS is known to be the production of a penicillin-binding protein peculiar to them, designated PBP2' or PBP2a, which has low affinity for commercially available β-lactams (8). Furthermore, most clinical isolates of MRSA and MRCNS have acquired resistance to not only β-lactams but also various other antibiotics used in clinical settings. The worldwide spread and increased frequency of occurrence of these pathogens have caused difficulties in therapeutic treatment with antibiotics (5, 25).

Some antibiotics such as vancomycin, linezolid, or dalfopristin-quinupristin show clinical efficacy against infections caused by methicillin-resistant staphylococci (1, 6, 14) but have antibacterial activity against only gram-positive bacteria. Much effort has been spent on the development of β-lactams with anti-MRSA activity in laboratories worldwide (10, 12, 24). Some compounds, for example, RWJ-54428 (MC-02479) or BMS-247243, have been reported elsewhere as anti-MRSA agents, but most of them also exhibit narrow-spectrum antibacterial activity (7, 13). On the other hand, broad-spectrum cephalosporins like ceftriaxone or cefepime have been useful against various infectious diseases but are not active against

methicillin-resistant staphylococci. BAL9141 (formerly Ro-63-9141), which is under development, has been reported elsewhere to have satisfactory broad-spectrum activity with anti-MRSA activity (9). However, it does not have good water solubility and requires esterification to improve its solubility. With the aim of developing a broad-spectrum cephalosporin with anti-MRSA activity, we selected S-3578, 7β-[2-(2-aminothiadiazol-4-yl)-2(Z)-ethoxyiminoacetamido]-3-(1-N-methylaminopropyl-1H-imidazo[4,5-b]pyridinium-4-yl)-methylcephalosporin, because of its antibacterial activities, aqueous solubility, and crystallinity (26).

In this study, we evaluated the *in vitro* antibacterial activity of S-3578, particularly focusing on its anti-MRSA activity.

(Part of this work was presented previously [Y. Yamano, H. Miwa, K. Motokawa, T. Yoshida, J. Shimada, and S. Kuwahara, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-371, 2001; T. Fujimura, Y. Yamano, I. Yoshida, T. Yoshida, J. Shimada, and S. Kuwahara, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-372, 2001].)

MATERIALS AND METHODS

Antibiotics. S-3578 was synthesized in the research laboratories of Shionogi & Co., Ltd. (Osaka, Japan). Its chemical structure is shown in Fig. 1. Other compounds were purchased from commercial sources.

Organisms. Bacterial strains used in this study were clinical isolates collected from hospitals in various parts of Japan in 2000 and 2001 and strains from our laboratory collection. *S. aureus* SRM710, deficient in PBP2, was used in a binding affinity assay of PBP2a of MRSA (18).

MIC determination. MICs for aerobic bacteria were determined by a microdilution broth method as recommended by the NCCLS (20) with cation-adjusted Mueller-Hinton broth (CA-MHB) (Becton Dickinson, Sparks, Md.), which was

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FIG. 1. Chemical structure of S-3578, 7 β -[2-(2-aminothiazol-4-yl)-2(Z)-ethoxyiminoacetamido]-3-(1-N-methylaminopropyl-1H-imidazo[4,5-b]pyridinium-4-yl)-methylcephalosporin.

supplemented with 15 μ g of NAD/ml, 5% yeast extract (Becton Dickinson), and 5% lysed horse blood to support the growth of *Streptococcus pneumoniae* and *Haemophilus influenzae*. CA-MHB with 2% NaCl was used to determine MICs of oxacillin for staphylococci. MICs for *Neisseria gonorrhoeae* were determined by an agar dilution method with GC medium (Becton Dickinson) containing growth supplement after incubation at 35°C for 20 h in the presence of 6% CO₂. MICs for anaerobic bacteria were determined by an agar dilution method with Wilkins-Chalgren medium (Becton Dickinson) after anaerobic incubation at 35°C for 48 h.

Determination of minimum bactericidal concentration (MBC). MBCs were determined by a macrodilution method with CA-MHB according to the NCCLS recommendations (19).

Time-kill study. The culture of a test strain at the early or mid-log phase was diluted to about 5×10^5 CFU/ml in 5 ml of fresh CA-MHB. Antibiotics at various concentrations were added to the diluted cultures. Viable cells were counted after shaking at 37°C for 0, 1, 2.5, 4, and 6 h. In the studies of *S. aureus*, the additional counting was performed at 24 h.

PBP binding affinity. The affinities of antibiotics for PBPs were determined by a competition assay with [¹⁴C]benzylpenicillin (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, England) as described previously (17, 23). The binding affinity of each compound was expressed as the concentration required to inhibit 50% of the binding of [¹⁴C]benzylpenicillin to each PBP (IC₅₀).

Population analysis and frequency of emergence of resistant colonies. An overnight culture of *S. aureus* in CA-MHB was diluted with fresh medium to the appropriate bacterial density and spread onto a Mueller-Hinton agar plate containing serial twofold dilutions of antibiotics. The plates were incubated for 3 days at 37°C, and the number of colonies was counted. The frequency of emergence of resistant colonies on an agar plate containing S-3578 was shown as their ratio to the number of colonies grown on an agar plate without antibiotics.

β -Lactamase stability. β -Lactamases were purified partially from bacterial membrane by ion-exchange chromatography as described previously (16). The stability of antibiotics against hydrolysis by β -lactamases was assessed by determining the hydrolysis rate by a spectrophotometric assay method (21). The kinetics parameters K_m and V_{max} were calculated from at least two independent experiments with the Michaelis-Menten formulation.

RESULTS

MIC determination. The antibacterial activity of S-3578 was compared with those of cefepime, ceftriaxone, ceftazidime, and imipenem. For gram-positive bacteria, vancomycin was also used as a comparator, as were oxacillin for staphylococci and penicillin G for *S. pneumoniae*. The results are shown in Table 1 as the MIC range, MIC₅₀ (the concentration at which the growth of 50% of the isolates was inhibited), and MIC₉₀.

S-3578 showed consistent antibacterial activity against staphylococci including methicillin-resistant strains and inhibited the growth of all clinical isolates of staphylococci at 8 μ g/ml. The MIC₉₀s of S-3578 for MRSA and methicillin-resistant *Staphylococcus epidermidis* (MRSE) were 4 and 2 μ g/ml, respectively, whereas those of other β -lactam compounds were 64 μ g/ml or more. S-3578 was fourfold less active against MRSA than was vancomycin, but its activity against MRSE

was equal to that of vancomycin. For methicillin-susceptible *S. aureus* (MSSA) and methicillin-susceptible *S. epidermidis*, the MIC₉₀s of S-3578 were 2 and 0.5 μ g/ml, respectively. It was characteristic that the MICs of S-3578 for methicillin-resistant staphylococcus strains were only two- to fourfold higher than those for methicillin-susceptible staphylococcus strains.

S-3578 had activities against penicillin-susceptible, -intermediate, and -resistant *S. pneumoniae*, with MIC₉₀s of 0.25, 1, and 1 μ g/ml, respectively, which were comparable to those of ceftriaxone and cefepime. However, S-3578 was not as active against *Enterococcus faecalis* as were other cephalosporins, though it showed the lowest MIC₅₀ among the cephalosporins tested.

S-3578 was also active against a variety of gram-negative bacteria, although its activity was not superior to those of cefepime and imipenem, except that imipenem was less active than was S-3578 against *H. influenzae*. The MIC₉₀s of S-3578 for *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* were 1 μ g/ml or less, which was comparable to those of ceftazidime but two- to eightfold higher than those of cefepime, ceftriaxone, and imipenem. Some S-3578-resistant strains of these species were also resistant to other cephalosporins. The MIC₉₀s of S-3578 for *Enterobacter aerogenes*, *Citrobacter freundii*, *Serratia marcescens*, *Proteus mirabilis*, and *Providencia rettgeri* ranged from 2 to 8 μ g/ml, and most strains of *Proteus vulgaris* were not susceptible to S-3578. S-3578 was active against *H. influenzae* except for β -lactamase-negative ampicillin-resistant strains with a MIC₉₀ of 0.25 μ g/ml, regardless of the β -lactamase production. Its activity was comparable to those of cefepime and ceftazidime but lower than that of ceftriaxone. The activity of S-3578 against *Moraxella catarrhalis* (MIC₉₀, 1 μ g/ml) was higher than those of ceftriaxone and cefepime but fourfold lower than that of ceftazidime. S-3578 showed activity against *Pseudomonas aeruginosa* with a MIC₅₀ and a MIC₉₀ of 8 and 64 μ g/ml, respectively, which were two- to fourfold higher than those of ceftazidime and cefepime. The activity of S-3578 against *Acinetobacter baumannii* was the highest among the cephalosporins tested, though its MIC₅₀ and MIC₉₀ were 1 and 16 μ g/ml, respectively. S-3578, however, had the same potential against *N. gonorrhoeae* as did penicillin G, though other compounds were more active.

The antibacterial activities against anaerobic pathogens were determined. S-3578 inhibited growth of all strains of *Peptostreptococcus* spp. at 8 μ g/ml, which meant that it was as active as ceftriaxone was. *Bacteroides fragilis* was not susceptible to S-3578.

TABLE 1. In vitro antibacterial activities of S-3578 and reference compounds against clinical isolates

Organism(s) (no. of isolates) and compound	MIC ($\mu\text{g/ml}$)			Organism(s) (no. of isolates) and compound	MIC ($\mu\text{g/ml}$)		
	Range	MIC ₅₀	MIC ₉₀		Range	MIC ₅₀	MIC ₉₀
MSSA (84)				<i>Staphylococcus warneri</i> (39)			
S-3578	0.5–2	1	2	S-3578	0.13–1	0.5	0.5
Ceftriaxone	1–8	4	4	Ceftriaxone	0.5–16	2	4
Cefepime	1–4	4	4	Cefepime	0.25–2	1	2
Ceftazidime	4–32	16	16	Ceftazidime	4–32	8	16
Imipenem	≤ 0.06 – ≤ 0.06	≤ 0.06	≤ 0.06	Imipenem	≤ 0.06 – ≤ 0.06	≤ 0.06	≤ 0.06
Vancomycin	0.5–2	1	1	Vancomycin	0.5–2	1	2
Oxacillin	0.25–2	0.5	1	Oxacillin	0.13–8	0.25	0.5
MRSA (134)				<i>Staphylococcus lugdunensis</i> (21)			
S-3578	1–8	4	4	S-3578	0.5–2	1	1
Ceftriaxone	8–>64	>64	>64	Ceftriaxone	4–64	4	4
Cefepime	8–>64	>64	>64	Cefepime	1–16	2	2
Ceftazidime	32–>64	>64	>64	Ceftazidime	8–64	16	16
Imipenem	≤ 0.06 –>64	64	64	Imipenem	≤ 0.06 –0.25	≤ 0.06	≤ 0.06
Vancomycin	0.5–2	1	1	Vancomycin	0.5–2	1	2
Oxacillin	8–>64	>64	>64	Oxacillin	0.25–>64	0.5	0.5
MSSE^a (28)				<i>Staphylococcus caprae</i> (8), <i>Staphylococcus hominis</i> (2), <i>Staphylococcus simulans</i> (1), <i>Staphylococcus schleifrei</i> (1), <i>Staphylococcus cohnii</i> (1), <i>Staphylococcus hyicus</i> (1)			
S-3578	≤ 0.06 –1	0.5	0.5	S-3578	0.13–1	0.25	1
Ceftriaxone	0.25–2	1	2	Ceftriaxone	1–>64	4	>64
Cefepime	0.13–2	0.5	1	Cefepime	0.25–>64	0.5	>64
Ceftazidime	2–8	4	8	Ceftazidime	4–>64	4	>64
Imipenem	≤ 0.06 – ≤ 0.06	≤ 0.06	≤ 0.06	Imipenem	≤ 0.06 –32	≤ 0.06	32
Vancomycin	0.5–2	1	2	Vancomycin	0.5–2	1	1
Oxacillin	≤ 0.06 –0.13	0.13	0.13	Oxacillin	0.13–>64	0.25	>64
MRSE (105)				<i>Streptococcus pyogenes</i> (60)			
S-3578	0.5–4	1	2	S-3578	≤ 0.03 – ≤ 0.03	≤ 0.03	≤ 0.03
Ceftriaxone	4–>64	32	>64	Ceftriaxone	≤ 0.03 – ≤ 0.03	≤ 0.03	≤ 0.03
Cefepime	2–>64	16	>64	Cefepime	≤ 0.03 –0.06	≤ 0.03	≤ 0.03
Ceftazidime	8–>64	32	>64	Ceftazidime	0.13–0.25	0.13	0.25
Imipenem	≤ 0.06 –>64	8	64	Imipenem	≤ 0.03 – ≤ 0.03	≤ 0.03	≤ 0.03
Vancomycin	1–2	1	2	Vancomycin	0.5–0.5	0.5	0.5
Oxacillin	1–>64	32	>64	<i>Streptococcus agalactiae</i> (68)			
<i>Staphylococcus haemolyticus</i>, methicillin susceptible (7)				S-3578	≤ 0.03 –0.06	0.06	0.06
S-3578	0.25–0.5			Ceftriaxone	≤ 0.03 –0.13	0.06	0.06
Ceftriaxone	8–32			Cefepime	0.06–0.13	0.06	0.13
Cefepime	1–4			Ceftazidime	0.25–1	0.5	0.5
Ceftazidime	8–64			Imipenem	≤ 0.03 – ≤ 0.03	≤ 0.03	≤ 0.03
Imipenem	≤ 0.06 – ≤ 0.06			Vancomycin	0.5–1	1	1
Vancomycin	0.5–1			<i>Streptococcus mitis</i> group (43)			
Oxacillin	0.13–0.25			S-3578	≤ 0.03 –>32	0.13	8
<i>Staphylococcus haemolyticus</i>, methicillin resistant (42)				Ceftriaxone	≤ 0.03 –16	0.13	8
S-3578	0.5–8	1	4	Cefepime	≤ 0.03 –>32	0.13	4
Ceftriaxone	16–>64	64	>64	Ceftazidime	0.06–>32	2	>32
Cefepime	4–>64	32	>64	Imipenem	≤ 0.03 –4	≤ 0.03	2
Ceftazidime	16–>64	>64	>64	Vancomycin	0.25–2	0.5	1
Imipenem	≤ 0.06 –>64	0.5	>64	<i>Streptococcus anginosus</i> group (48)			
Vancomycin	1–4	2	4	S-3578	0.13–0.5	0.25	0.5
Oxacillin	0.5–>64	>64	>64	Ceftriaxone	0.06–0.5	0.25	0.25
<i>Staphylococcus capitis</i> (25)				Cefepime	0.13–1	0.5	0.5
S-3578	0.13–2	1	1	Ceftazidime	1–8	4	8
Ceftriaxone	0.5–>64	64	>64	Imipenem	≤ 0.03 –0.06	≤ 0.03	≤ 0.03
Cefepime	0.25–>64	32	>64	Vancomycin	0.5–1	1	1
Ceftazidime	2–>64	64	>64	<i>Streptococcus pneumoniae</i>, peni- cillin susceptible (61)			
Imipenem	≤ 0.06 –64	1	64	S-3578	≤ 0.03 –0.5	0.06	0.25
Vancomycin	0.5–2	1	2	Ceftriaxone	≤ 0.03 –0.5	0.13	0.25
Oxacillin	≤ 0.06 –>64	>64	>64	Cefepime	≤ 0.03 –1	0.13	0.5
<i>Staphylococcus saprophyticus</i> (30)				Ceftazidime	0.06–16	2	8
S-3578	0.5–2	1	1	Imipenem	≤ 0.03 –0.06	≤ 0.03	≤ 0.03
Ceftriaxone	8–>64	16	32	Vancomycin	0.25–0.5	0.5	0.5
Cefepime	2–>64	2	4	Penicillin G	≤ 0.03 –0.06	≤ 0.03	0.06
Ceftazidime	32–>64	32	64				
Imipenem	≤ 0.06 –1	≤ 0.06	≤ 0.06				
Vancomycin	1–2	1	2				
Oxacillin	0.5–>64	1	2				

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TABLE 1—Continued

Organism(s) (no. of isolates) and compound	MIC (μg/ml)			Organism(s) (no. of isolates) and compound	MIC (μg/ml)		
	Range	MIC ₅₀	MIC ₉₀		Range	MIC ₅₀	MIC ₉₀
<i>Streptococcus pneumoniae</i> , peni- cillin intermediate (53)				<i>Citrobacter freundii</i> (42)			
S-3578	≤0.03–2	0.5	1	S-3578	0.13–16	0.25	8
Ceftriaxone	≤0.03–4	0.5	1	Ceftriaxone	≤0.06–>64	0.25	>64
Cefepime	0.13–2	0.5	1	Cefepime	≤0.06–2	≤0.06	1
Ceftazidime	0.25–16	8	16	Ceftazidime	0.13–>64	0.5	>64
Imipenem	≤0.03–0.13	0.06	0.13	Imipenem	≤0.06–1	0.25	0.5
Vancomycin	0.25–0.5	0.25	0.5	<i>Serratia marcescens</i> (93)			
Penicillin G	0.13–1	0.5	1	S-3578	0.13–>64	0.5	8
<i>Streptococcus pneumoniae</i> , peni- cillin resistant (35)				Ceftriaxone	≤0.06–>64	0.25	32
S-3578	0.5–2	1	1	Cefepime	≤0.06–>64	0.13	2
Ceftriaxone	0.5–2	1	1	Ceftazidime	0.13–>64	0.25	4
Cefepime	0.5–2	1	2	Imipenem	0.13–16	0.5	2
Ceftazidime	8–32	8	16	<i>Proteus mirabilis</i> (62)			
Imipenem	0.13–0.5	0.25	0.25	S-3578	0.5–>64	2	4
Vancomycin	0.25–0.5	0.5	0.5	Ceftriaxone	≤0.06–>64	≤0.06	≤0.06
Penicillin G	2–4	2	2	Cefepime	≤0.06–>64	≤0.06	≤0.06
<i>Enterococcus faecalis</i> (61)				Ceftazidime	≤0.06–0.25	≤0.06	0.13
S-3578	4–>64	16	>64	Imipenem	0.5–8	2	4
Ceftriaxone	64–>64	>64	>64	<i>Proteus vulgaris</i> (46)			
Cefepime	16–>64	64	>64	S-3578	1–>64	8	64
Ceftazidime	>64–>64	>64	>64	Ceftriaxone	≤0.06–>64	>64	>64
Imipenem	0.5–8	1	2	Cefepime	≤0.06–8	0.13	0.5
Vancomycin	0.5–4	1	2	Ceftazidime	≤0.06–2	≤0.06	0.25
<i>Peptostreptococcus magnus</i> (4), <i>Peptostreptococcus asaccha-</i> <i>rolyticus</i> (4), <i>Peptostreptococ-</i> <i>cus hydrogenalis</i> (3), <i>Pepto-</i> <i>streptococcus micros</i> (7)				Imipenem	0.5–8	2	4
S-3578	0.06–8	0.13	4	<i>Morganella morganii</i> (59)			
Ceftriaxone	0.06–8	0.13	2	S-3578	0.25–32	0.5	16
Cefepime	0.06–16	0.25	8	Ceftriaxone	≤0.06–8	≤0.06	4
Ceftazidime	0.25–32	1	8	Cefepime	≤0.06–0.25	≤0.06	≤0.06
Imipenem	≤0.016–0.06	≤0.016	0.03	Ceftazidime	≤0.06–32	0.5	16
Vancomycin	0.13–1	0.5	1	Imipenem	0.5–4	2	4
<i>Escherichia coli</i> (164)				<i>Providencia rettgeri</i> (27)			
S-3578	0.06–>64	0.25	0.5	S-3578	≤0.06–>64	0.25	8
Ceftriaxone	≤0.016–>64	0.06	0.13	Ceftriaxone	≤0.06–>64	≤0.06	>64
Cefepime	≤0.016–>64	0.03	0.13	Cefepime	≤0.06–>64	≤0.06	2
Ceftazidime	0.06–>64	0.13	0.5	Ceftazidime	≤0.06–>64	0.13	>64
Imipenem	0.06–2	0.13	0.25	Imipenem	0.5–2	1	2
<i>Klebsiella pneumoniae</i> (87)				<i>Haemophilus influenzae</i> , ampicil- lin susceptible (56)			
S-3578	≤0.06–>64	0.25	1	S-3578	≤0.06–0.25	0.13	0.25
Ceftriaxone	≤0.06–>64	≤0.06	0.13	Ceftriaxone	≤0.06–0.13	≤0.06	≤0.06
Cefepime	≤0.06–16	≤0.06	0.13	Cefepime	≤0.06–0.25	≤0.06	0.13
Ceftazidime	≤0.06–4	0.13	0.5	Ceftazidime	≤0.06–0.5	0.13	0.25
Imipenem	≤0.06–0.5	0.13	0.25	Imipenem	0.13–4	1	2
<i>Klebsiella oxytoca</i> (62)				Ampicillin	0.13–1	0.25	0.5
S-3578	≤0.06–8	0.13	1	<i>Haemophilus influenzae</i> , β-lacta- mase producing (7)			
Ceftriaxone	≤0.06–64	≤0.06	0.13	S-3578	≤0.06–0.5	≤0.06–0.5	≤0.06
Cefepime	≤0.06–2	≤0.06	0.13	Ceftriaxone	≤0.06–0.25	≤0.06–0.25	≤0.06
Ceftazidime	≤0.06–1	0.13	0.5	Cefepime	≤0.06–0.5	0.13	0.25
Imipenem	≤0.06–2	0.13	0.25	Imipenem	0.13–2	1	2
<i>Enterobacter cloacae</i> (84)				Ampicillin	4–>64		
S-3578	≤0.06–>64	0.5	16	<i>Haemophilus influenzae</i> , BLNAR ^b (37)			
Ceftriaxone	≤0.06–>64	0.5	>64	S-3578	0.25–8	2	8
Cefepime	≤0.06–32	≤0.06	1	Ceftriaxone	≤0.06–0.5	≤0.06	0.25
Ceftazidime	≤0.06–>64	0.5	64	Cefepime	≤0.06–2	0.5	2
Imipenem	≤0.06–4	1	2	Ceftazidime	≤0.06–1	0.25	0.5
<i>Enterobacter aerogenes</i> (51)				Imipenem	0.25–64	4	16
S-3578	≤0.06–8	0.25	2	Ampicillin	2–32	2	16
Ceftriaxone	≤0.06–>64	0.25	32	<i>Moraxella catarrhalis</i> (53)			
Cefepime	≤0.06–4	≤0.06	0.5	S-3578	≤0.06–4	0.5	1
Ceftazidime	0.13–>64	0.5	64	Ceftriaxone	≤0.06–32	0.5	2
Imipenem	0.13–2	1	2	Cefepime	≤0.06–16	1	2
				Ceftazidime	≤0.06–8	0.13	0.25
				Imipenem	≤0.06–0.13	≤0.06	0.13

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TABLE 1—Continued

Organism(s) (no. of isolates) and compound	MIC ($\mu\text{g/ml}$)			Organism(s) (no. of isolates) and compound	MIC ($\mu\text{g/ml}$)		
	Range	MIC ₅₀	MIC ₉₀		Range	MIC ₅₀	MIC ₉₀
<i>Pseudomonas aeruginosa</i> (108)				Cefepime	0.5–>64	4	32
S-3578	0.5–>64	8	64	Ceftazidime	2–>64	8	32
Ceftriaxone	2–>64	64	>64	Imipenem	0.13–64	0.25	0.25
Cefepime	0.5–>64	4	16	<i>Neisseria gonorrhoeae</i> (21)			
Ceftazidime	1–>64	2	32	S-3578	0.06–>64	4	32
Imipenem	≤ 0.06 –>64	1	16	Ceftriaxone	0.008–0.5	0.06	0.25
<i>Burkholderia cepacia</i> (21)				Cefepime	0.016–16	0.5	8
S-3578	32–>64	>64	>64	Ceftazidime	0.06–4	1	2
Ceftriaxone	16–>64	64	>64	Imipenem	0.06–2	0.5	2
Cefepime	16–>64	32	>64	Penicillin G	0.25–>64	4	16
Ceftazidime	2–32	4	16	<i>Bacteroides fragilis</i> (41)			
Imipenem	4–32	16	16	S-3578	4–>128	64	>128
<i>Acinetobacter baumannii</i> (40)				Ceftriaxone	4–>128	16	128
S-3578	0.13–>64	1	16	Cefepime	4–>128	64	>128
Ceftriaxone	8–>64	16	64	Ceftazidime	4–>128	32	>128
				Imipenem	0.06–4	0.13	1

^a MSSE, methicillin-susceptible *S. epidermidis*.

^b BLNAR, β -lactamase-negative ampicillin-resistant strain.

Bactericidal activity. We first determined MBCs for 20 clinical isolates each of MRSA and MSSA including β -lactamase producers. The MBC/MIC ratios for S-3578 against all strains were 1 or 2 (Fig. 2). In particular, the number of MSSA strains showing the ratio of 1 for S-3578 was larger than the number of those showing the same ratio for cefepime and ceftriaxone, suggesting that S-3578 was more bactericidal than the reference agents were. The production of β -lactamase did not have any influence on both MBCs and MICs of S-3578 as well as of the reference compounds.

Next, we constructed the killing kinetics curves for MRSA and MSSA strains. S-3578 caused a time-dependent decrease of viable cells of the MSSA strain Smith at one or more times the MIC until 6 h, as did cefepime and ceftriaxone (Fig. 3B). Furthermore, the number of viable cells after 24-h exposure to S-3578 was significantly lower than the number after exposure to the reference compounds. S-3578 also showed similar bactericidal activity against the MRSA strain SR3637 at two or more times the MIC (Fig. 3A). Moreover, the decreases of viable cells with 10 clinical isolates each of MRSA or MSSA were compared after 24-h exposure to twice the MIC of each antibiotic. S-3578 caused more than a $4\text{-log}_{10}\text{-CFU/ml}$ reduction on the average for both MRSA and MSSA, while cefepime and ceftriaxone demonstrated only less than a $2\text{-log}_{10}\text{-CFU/ml}$ reduction for MSSA (Fig. 4). These results indicated that the bactericidal activity of S-3578 was more potent than those of cefepime and ceftriaxone against *S. aureus*, irrespective of methicillin resistance.

The MBCs of S-3578 for 20 clinical isolates of *E. coli* for which MICs were 0.13 to 1 $\mu\text{g/ml}$ ranged from 0.25 to 1 $\mu\text{g/ml}$, while the MBCs and MICs of cefepime ranged from 0.25 to 2 $\mu\text{g/ml}$ and from 0.13 to 2 $\mu\text{g/ml}$, respectively (Fig. 5). All strains tested were β -lactamase producers, indicating that the activity of S-3578 was not affected by β -lactamase. Furthermore, the killing kinetics curves for *E. coli* NIHJ JC-2 and *P. aeruginosa* SR24706 indicated that S-3578 also had time-dependent bactericidal activity against these species which was comparable to those of the reference compounds (Fig. 6).

Binding affinity for PBPs. S-3578 was found to have high affinity for PBP2a of MRSA, with an IC₅₀ of 4.5 $\mu\text{g/ml}$ (Table 2). This characteristic seemed to reflect the anti-MRSA activity of S-3578. In contrast, cefepime, which was not active against MRSA, had a very low affinity for PBP2a. S-3578 also showed high affinities for PBP1 and -2 of *S. aureus* with IC₅₀s of less than 0.5 $\mu\text{g/ml}$, as did ceftriaxone and cefepime.

Cefepime had high affinities for PBP2 and -3 of *E. coli* with

FIG. 2. Distribution of MBCs and MICs for 20 clinical isolates each of MRSA (A) or MSSA (B). The activity of S-3578 was compared with those of cefepime and ceftriaxone against MSSA. Open and closed circles indicate β -lactamase-producing and nonproducing strains, respectively.

A

B

FIG. 3. Killing kinetics curves for S-3578 and reference compounds against *S. aureus* SR3637 (MRSA) (A) and Smith (MSSA) (B).

IC_{50} s of less than 1 µg/ml, and ceftriaxone had affinities for PBP1A, -1B, -2, and -3 (Table 3). On the other hand, S-3578 had high affinity only for PBP3. Although the IC_{50} s of S-3578 for PBP1A, -3, and -4 of *P. aeruginosa* ranged from 0.31 to 0.43

µg/ml (Table 4), its binding affinity was not as high as those of cefepime and ceftazidime, the IC_{50} s of which for PBP3 were 10-fold less than that of S-3578.

Frequency of emergence of resistant colonies. In order to estimate the frequency of resistance development, we first carried out population analysis on two clinical isolates of MRSA,

FIG. 4. Decrease of viable cells in 10 clinical isolates each of MRSA or MSSA after 24-h exposure to twice the MIC of S-3578. Cefepime and ceftriaxone were used as comparators for MSSA. An open circle indicates decrease of viable cell counts of each strain compared to the initial inoculum, and a bar indicates the average of those of 10 strains.

FIG. 5. Distribution of MBCs and MICs for 20 clinical isolates of *E. coli*. The activity of S-3578 was compared with that of cefepime. All strains produced β -lactamases.

FIG. 6. Killing kinetics curves for S-3578 and reference compounds against *E. coli* NIHJ JC-2 (A) and *P. aeruginosa* SR24706 (B).

the low-level-methicillin-resistant strain SR19754 and the high-level-methicillin-resistant strain SR19760. As shown in Fig. 7, strain SR19754 had a subpopulation resistant to imipenem at a frequency of about 10^{-6} in spite of the low MIC of imipenem for this strain, that is, it exhibited a heteroresistance profile. In contrast, the viabilities of both strains against S-3578 sharply decreased with increase of the antibiotic concentration. In particular, it was notable that no colonies emerged on agar plates containing twice the MIC of S-3578. Furthermore, about 10^9 bacterial cells each of 20 clinical isolates of MRSA were spread on agar plates containing S-3578 and incubated for 3 days at 35°C. For all isolates, no colonies appeared on the plates at twice the MIC, and emergence from an isolate for

which the MIC was 8 µg/ml did not occur even at the MIC (data not shown). From these results, the frequency at which resistant colonies emerged was estimated to be below 10^{-9} .

β-Lactamase stability. The hydrolysis of S-3578 and cefepime by a β-lactamase purified from *S. aureus* SR5644 was not detected at 100 µg of the compounds per ml (data not shown), indicating that S-3578 was stable against hydrolysis by the β-lactamase from *S. aureus*. S-3578, as well as cefepime, was stable against TEM-1 and a class A β-lactamase from *K. pneumoniae* GN69 (22) because their hydrolysis rates were less than 0.1% of that of ampicillin at the substrate concentration of 100 µg/ml (data not shown). Although the K_m values of S-3578 against various AmpC enzymes from *C. freundii*, *Enterobacter cloacae*, and *P. aeruginosa* were lower than those of cefepime,

TABLE 2. Binding affinity for PBPs of *S. aureus*

Compound	IC ₅₀ (µg/ml) ^a					MIC (µg/ml)	
	PBP1	PBP2a	PBP2	PBP3	PBP4	Smith	SRM710
S-3578	0.28	4.5	0.34	4.2	16	1	2
Cefepime	0.37	410	0.12	>16	>16	2	64
Ceftriaxone	0.31	NT ^b	0.11	2.0	>16	4	NT

^a IC₅₀ was determined by a competition assay with the membrane fraction of *S. aureus* SRM710 (MRSA) for PBP2a and that of *S. aureus* Smith (MSSA) for other PBPs.

^b NT, not tested.

TABLE 3. Binding affinity for PBPs of *E. coli*

Compound	IC ₅₀ (µg/ml)							MIC (µg/ml) ^a
	PBP1A	PBP1B	PBP2	PBP3	PBP4	PBP5	PBP6	
S-3578	5.8	4.3	>16	0.18	>16	>16	>16	0.25
Cefepime	>4	3.4	0.29	0.021	>4	>4	>4	0.03
Ceftriaxone	0.092	0.24	0.30	0.013	>4	>4	>4	0.06

^a MIC for *E. coli* NIHJ JC-2, the membrane fraction of which was employed for determination of IC₅₀s.

TABLE 4. Binding affinity for PBPs of *P. aeruginosa*

Compound	IC ₅₀ (μg/ml)							MIC (μg/ml) ^a
	PBP1A	PBP1B	PBP2	PBP3	PBP4	PBP5	PBP6	
S-3578	0.34	5.0	>16	0.31	0.43	>16	>16	1
Cefepime	0.076	7.6	>16	0.024	0.79	>16	>16	0.5
Ceftazidime	0.11	5.1	5.3	0.022	1.6	>16	>16	1

^a MIC for *P. aeruginosa* ATCC 25619, the membrane fraction of which was employed for determination of IC₅₀s.

its relative $V_{\max}/K_m$ values were comparable to those of cefepime. These results indicated that S-3578 had higher affinity for AmpC enzymes but was as stable as cefepime was against hydrolysis (Table 5). On the other hand, S-3578 was not as stable as cefepime was against a Toho-1-like extended-spectrum β -lactamase and a class B IMP-1-like enzyme (data not shown).

DISCUSSION

One of the most striking features of S-3578 was its activity against methicillin-resistant staphylococci. S-3578 inhibited growth of all clinical isolates of *Staphylococcus* spp. including methicillin-resistant strains at 8 μg/ml, although other β -lactam compounds including imipenem were not active against most strains of methicillin-resistant staphylococci. We further characterized the anti-MRSA activity of S-3578 as follows. First, this activity of S-3578, like those of other anti-MRSA β -lactams (3, 9, 15), was considered to be due to the high affinity for PBP2a. This was consistent with reports of a correlation between the affinity for PBP2a and the MIC for MRSA strains with a high level of resistance to β -lactams (2, 11). Second, S-3578 showed potent bactericidal activity against MSSA and MRSA strains. The superiority of S-3578 over cefepime and ceftazidime in bactericidal activity against MSSA was represented by a significant decrease of viable cells after 24-h exposure to S-3578. The results in Fig. 3 and 4 indicate that S-3578 had potent bactericidal activity against not only MSSA but also MRSA. Third, the frequency of emergence of resistant colonies from MRSA strains was extremely low. Moreover, S-3578 showed remarkable reduction of the viability of the heteroresistant strain in contrast with imipenem, which easily

TABLE 5. Stability of S-3578 against AmpC β -lactamases

Enzyme source	Compound	K _m (μM)	Relative V _{max} ^a	Relative V _{max} /K _m ^a
<i>Citrobacter freundii</i> SR19	S-3578	26	0.008	0.20
	Cefepime	340	0.030	0.056
	Cephaloridine	640	100	100
<i>Enterobacter cloacae</i> SR4321	S-3578	19	0.006	0.16
	Cefepime	500	0.14	0.14
	Cephaloridine	500	100	100
<i>Pseudomonas aeruginosa</i> SR24-12	S-3578	60	0.030	0.35
	Cefepime	560	0.11	0.14
	Cephaloridine	700	100	100

^a The value is expressed relative to an arbitrary value of 100 for cephaloridine.

selected its resistant subpopulation (Fig. 7). These results suggested that S-3578-resistant strains would rarely emerge during antibiotic treatment. The two latter features would contribute to in vivo therapeutic efficacy against MRSA infection.

In addition to the activity against methicillin-resistant staphylococci, S-3578 was highly active against streptococci including penicillin-resistant *S. pneumoniae*, though most *E. faecalis* strains were not susceptible to this compound. On the other hand, S-3578 was not as active against gram-negative bacteria as cefepime was, but it showed antibacterial activity comparable to that of ceftazidime against most members of the family *Enterobacteriaceae*, *H. influenzae*, and *M. catarrhalis*. The anti-pseudomonal activity of S-3578, with a MIC₅₀ of 8 μg/ml, was two- to fourfold less than those of ceftazidime and cefepime.

In conclusion, S-3578 is a novel parenteral broad-spectrum cephalosporin antibiotic with high activity against methicillin-resistant staphylococci. It should be noted that S-3578 is soluble in water at more than 100 mg/ml (26). This water solubility is a great advantage because most anti-MRSA cephalosporins have been reported elsewhere to be insoluble in water (4, 9). The pharmacokinetic profile of S-3578 in mice is similar to that of cefepime, and S-3578 shows good therapeutic efficacy in murine models of infection by MRSA, penicillin-resistant *S. pneumoniae*, or *P. aeruginosa* (M. Tsuji, M. Takema, H. Miwa, J. Shimada, and S. Kuwahara, submitted for publication). Thus, S-3578 is considered to be a promising compound

FIG. 7. Population analysis profiles of two clinical isolates of MRSA. Strains SR19754 (A) and SR19760 (B) are low-level- and high-level-methicillin-resistant strains, respectively. The MICs of S-3578 and imipenem for each strain are indicated in the figure. Closed circles and open triangles indicate viable cell counts on plates containing S-3578 or imipenem, respectively.

for further evaluation as an anti-MRSA broad-spectrum cephalosporin antibiotic.

ACKNOWLEDGMENTS

We are grateful to M. Doi, Y. Jinushi, Y. Kimura, H. Motoyama, K. Motokawa, T. Muneke, and K. Uotani for their superb technical assistance.

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