ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1985, p. 684–688
0066-4804/85/110684-05$02.00/0
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Vol. 28, No. 5

In Vitro Antibacterial Activity of Sch 34343 and Its Stability to β-Lactamases and Renal Dehydropeptidase 1

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Received 20 March 1985/Accepted 24 August 1985

The in vitro antibacterial activity of Sch 34343 against 1,328 strains of clinical isolates was compared with those of imipenem and ceftazidime. Sch 34343 had a broad spectrum of antibacterial activity against both gram-positive and gram-negative bacteria but was inactive against *Pseudomonas aeruginosa* and *Pseudomonas maltophilia*. Sch 34343 was quite stable to hydrolysis by β-lactamases, including both penicillinases and cephalosporinases. However, Sch 34343 was slightly hydrolyzed by a new type of β-lactamase (oxyiminocephalosporin β-lactamase), as was imipenem. Sch 34343 was slightly hydrolyzed by renal dehydropeptidase 1 but was somewhat more stable than other carbapenems.

Many kinds of β-lactam antibiotics have been used clinically for the treatment of a wide range of bacterial infections. Recently, however, the effectiveness of the existing β-lactam antibiotics has been shown to be limited by a speedy increase in clinical infections caused by bacteria resistant to β-lactam antibiotics (13). Much effort has been expended in developing broad-spectrum antibiotics with resistance to β-lactamases. Recently, nontraditional β-lactam antibiotics such as carbapenems (5), penems (3) and monobactams (19) have been developed.

Sch 34343, a new penem antibiotic, was developed by Schering Corp., Bloomfield, N.J. (15). Another penem, Sch 29482 (3), was developed before Sch 34343. The current report summarizes the results of studies of the in vitro antibacterial activity, β-lactamase stability, and dehydropeptidase 1 stability of Sch 34343.

MATERIALS AND METHODS

Antibiotics. Sch 34343 and Sch 29482 were obtained from Schering Corp. As comparative compounds, imipenem, ceftazidime (CAZ), aztreonam, and carpetimycin A and B were supplied by Merck Sharp & Dohme, Rahway, N.J., Glaxo Inc., Research Triangle Park, N.C., E. R. Squibb & Sons, Princeton, N.J., and Kowa Co., Ltd., Tokyo, respectively.

Organisms. A large number of gram-positive and gram-negative bacteria were recent clinical isolates from several hospitals. β-Lactamase-producing strains used herein were selected from our stocked standard cultures (10). All these organisms were maintained in glycerine broth at −80°C.

Media. Sensitivity disk agar, sensitivity test broth, Gifu anaerobic medium agar, Gifu anaerobic medium broth (Nissui Pharmaceutical Co., Ltd., Tokyo), brain heart infusion agar (Difco Laboratories, Detroit, Mich.), and nutrient agar containing bromothymol blue and lactose were used. For the dilution of cell suspensions, buffered saline gelatin (contains [grams per liter]: NaCl, 8.5; KH_2PO_4, 0.3; NaH_2PO_4, 0.6; gelatin, 0.1) was used.

Determination of MICs. MICs were determined by a twofold serial agar dilution method. For *Bacteroides fragilis* and streptococci, Gifu anaerobic medium agar and sensitivity disk agar supplemented with 5% defibrinated horse blood were used, respectively. For *Haemophilus influenzae*, sensitivity disk agar containing 10 μg of hemin and 5 μg of β-NAD per ml was used. All antibiotic solutions were freshly made on the day of use. The test organism was grown overnight at 37°C in sensitivity test broth for preculture, unless otherwise specified. The other media used were as follows: sensitivity test broth containing 0.4% potassium nitrate for *Pseudomonas aeruginosa*, brain heart infusion agar for streptococci, sensitivity test broth supplemented with 10 μg of hemin and 2 μg of β-NAD per ml for *H. influenzae*, and Gifu anaerobic medium broth for *B. fragilis*. Overnight cultures of tested strains were diluted, and about 3×10^4 CFU was applied to the agar surface with an inoculator (Microplanter; Sakuma Seisakusho, Tokyo). Plates were incubated for 18 h at 37°C. *B. fragilis* was incubated for 24 h at 37°C under anaerobic conditions. The MIC was defined as the lowest concentration of the antibiotic that prevented visible growth.

Stability to β-lactamase. Various types of β-lactamase (10) used in this study were purified by the methods described previously (2, 4, 6, 7, 9, 14, 16–18, 20). β-Lactamase activity was determined by the spectrophotometric method (21). For determining the rate of hydrolysis, the molecular absorbancy difference ($\Delta\varepsilon$) and the specific wavelength for Sch 34343 were 67,450 M^{-1} cm^{-1} and 330 nm, respectively. For other substrates, $\Delta\varepsilon$ and the specific wavelength were described previously (11). Enzymatic reaction was carried out at 30°C in a waterjacketed spectrophotometer (model 24; Beckman Instruments, Inc., Fullerton, Calif.).

The Michaelis constant (K_m) and maximum rate of hydrolysis (relative V_{max}) were determined from Lineweaver-Burk plots. The dissociation constant for the enzyme-inhibitor complex (K_i) was determined from Dixon plots.

Stability to renal dehydropeptidase 1. Renal dehydropeptidase 1 was purified from hog kidney cortex by the procedure described previously (8). Hydrolysis of the compounds was assayed spectrophotometrically (1) by measuring the decrease in absorbance at the substrate-specific wavelength in a temperature-controlled spectrophotometer

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TABLE 1. Antibacterial activity of Sch 34343 against clinical isolates

Organism (no. of strains)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Staphylococcus aureus (99)	Sch 34343	0.05–0.2	0.05	0.05
	Imipenem	0.006–0.1	0.006	0.013
	CAZ	3.13–25	3.13	3.13
Staphylococcus epidermidis (100)	Sch 34343	0.013–>100	0.1	6.25
	Imipenem	0.006–100	0.025	0.39
	CAZ	0.025–>100	6.25	12.5
Streptococcus pyogenes (53)	Sch 34343	0.013–0.39	0.025	0.05
	Imipenem	0.0015–0.006	0.0015	0.003
	CAZ	0.006–0.2	0.05	0.05
Streptococcus faecalis (54)	Sch 34343	1.56–25	3.13	3.13
	Imipenem	0.39–6.25	0.39	0.78
	CAZ	50–>100	>100	>100
Escherichia coli (94)	Sch 34343	0.1–0.78	0.2	0.2
	Imipenem	0.05–0.2	0.05	0.05
	CAZ	0.025–0.78	0.05	0.1
Salmonella spp. (49)	Sch 34343	0.2–0.39	0.2	0.2
	Imipenem	0.1–0.2	0.2	0.2
	CAZ	0.1–0.39	0.1	0.2
Shigella spp. (53)	Sch 34343	0.2–0.39	0.1	0.2
	Imipenem	0.05–0.2	0.05	0.1
	CAZ	0.025–0.39	0.025	0.1
Klebsiella pneumoniae (50)	Sch 34343	0.39–3.13	0.39	0.78
	Imipenem	0.05–0.39	0.1	0.2
	CAZ	0.025–3.13	0.05	0.2
Klebsiella oxytoca (27)	Sch 34343	0.39–0.78	0.2	0.39
	Imipenem	0.1–1.56	0.1	0.05
	CAZ	0.013–0.1	0.013	0.05
Serratia marcescens (53)	Sch 34343	0.39–100	0.78	6.25
	Imipenem	0.1–1.56	0.2	0.78
	CAZ	0.05–6.25	0.1	0.78
Enterobacter cloacae (96)	Sch 34343	0.2–6.25	0.39	0.78
	Imipenem	0.05–1.56	0.1	0.2
	CAZ	0.05–>100	0.39	25
Citrobacter freundii (57)	Sch 34343	0.2–6.25	0.2	0.78
	Imipenem	0.05–1.56	0.1	0.2
	CAZ	0.05–>100	0.2	12.5
Morganella morganii (91)	Sch 34343	0.39–6.25	0.78	1.56
	Imipenem	0.2–6.25	0.78	3.13
	CAZ	0.013–>100	0.05	0.39
Proteus mirabilis (77)	Sch 34343	0.1–0.78	0.2	0.39
	Imipenem	0.05–3.13	0.39	1.56
	CAZ	0.025–0.1	0.025	0.05
Proteus vulgaris (29)	Sch 34343	0.2–1.56	0.2	0.78
	Imipenem	0.2–6.25	0.78	1.56
	CAZ	0.025–0.2	0.025	0.1
Providencia rettgeri (21)	Sch 34343	0.2–1.56	0.2	0.39
	Imipenem	0.2–1.56	0.2	0.78
	CAZ	0.025–1.56	0.05	0.39
Acinetobacter spp. (49)	Sch 34343	0.2–12.5	1.56	3.13
	Imipenem	0.025–0.78	0.05	0.1
	CAZ	0.39–25	3.13	12.5
Pseudomonas aeruginosa (98)	Sch 34343	50–>200	100	200
	Imipenem	0.2–12.5	0.78	0.78
	CAZ	0.39–25	0.78	3.13

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TABLE 1—*Continued*

Organism (no. of strains)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Pseudomonas maltophilia (22)	Sch 34343	50->100	100	>100
	Imipenem	50->100	100	>100
	CAZ	0.78->100	3.13	12.5
Pseudomonas cepacia (51)	Sch 34343	0.39-6.25	3.13	3.13
	Imipenem	0.39-12.5	3.13	3.13
	CAZ	0.39->100	0.78	1.56
Haemophilis influenzae (68)	Sch 34343	0.013->100	0.2	0.78
	Imipenem	0.013-0.78	0.05	0.2
	CAZ	0.013-0.2	0.025	0.05
Neisseria gonorrhoeae (16)	Sch 34343	0.1-0.39	0.1	0.2
	Imipenem	0.78-6.25	1.56	3.13
	CAZ	0.013-0.2	0.05	0.05
Bacteroides fragilis (21)	Sch 34343	0.025-0.78	0.025	0.2
	Imipenem	0.05-6.25	0.05	0.78
	CAZ	3.13->200	6.25	200

[a] MIC_{50} and MIC_{90} are drug concentrations by which 50 and 90% of the isolates were inhibited, respectively.

(Beckman model 24). Glycyldehydrophenylalanine was used as the reference substrate. The specific wavelength of Sch 34343 was 330 nm, and those of other substrates were described in a previous paper (8). For the substrates of dehydropeptidase 1, the final concentration (100 μM) of glycyldehydrophenylalanine, Sch 34343, Sch 29482, imipenem, carpetimycin A and B, and aztreonam was used. All reactions were carried out in 50 mM MOPS [3-(*N*-morpholino)propanesulfonic acid] buffer (pH 7.2) at 35°C.

TABLE 2. Effect of inoculum sizes on the MICs of Sch 34343, imipenem, and ceftazidime

Organism	Inoculum size (CFU/spot)	MIC (μg/ml) of:		
		Sch 34343	Imipenem	Ceftazidime
Staphylococcus aureus FDA209P JC-1	1.0×10^5	0.1	0.025	12.5
	1.0×10^4	0.1	0.025	12.5
	1.0×10^3	0.1	0.025	12.5
	1.0×10^2	0.1	0.025	6.25
Escherichia coli K-12 C600	3.2×10^5	0.39	0.39	0.2
	3.2×10^4	0.2	0.2	0.1
	3.2×10^3	0.39	0.2	0.1
	3.2×10^2	0.2	0.2	0.1
Serratia marcescens IAM 1184	4.9×10^5	1.56	0.78	0.025
	4.9×10^4	1.56	0.2	0.025
	4.9×10^3	0.78	0.2	0.025
	4.9×10^2	0.78	0.1	0.025
Enterobacter cloacae 963	1.1×10^6	1.56	3.12	0.2
	1.1×10^5	0.39	0.78	0.2
	1.1×10^4	0.39	0.2	0.2
	1.1×10^3	0.2	0.2	0.2
Morganella morganii IFO3848	4.3×10^5	1.56	1.56	0.013
	4.3×10^4	0.78	0.39	0.013
	4.3×10^3	0.78	0.39	0.013
	4.3×10^2	0.78	0.39	0.013

RESULTS

In vitro antibacterial activity. The in vitro antibacterial activities of Sch 34343 against 1,328 clinical isolates of bacteria are shown in Table 1. Sch 34343 exhibited a broad spectrum of antibacterial activity against both gram-positive and gram-negative bacteria but showed no antibacterial activity against *Pseudomonas aeruginosa* and *Pseudomonas maltophilia*. Sch 34343 exhibited activities almost equal to those of imipenem against most species except *Pseudomonas aeruginosa*. *Staphylococcus aureus* strains were completely inhibited by Sch 34343 at a concentration of <0.2 μg/ml, whereas 50% of *Staphylococcus epidermidis* strains were inhibited by the same concentration. Over 90% of *Streptococcus pyogenes* strains were inhibited by 0.1 μg of Sch 34343 per ml. Sch 34343 showed medium antibacterial activity against *Streptococcus faecalis* strains.

In the case of members of the family *Enterobacteriaceae*, Sch 34343 showed excellent antibacterial activity. *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Klebsiella oxytoca*, *Proteus mirabilis*, *Proteus vulgaris*, and *Providencia rettgeri* were inhibited by Sch 34343 at a concentration of <1.56 μg/ml. Higher concentrations (6.25 μg/ml) of Sch 34343 were required to inhibit the strains of *Enterobacter cloacae*, *Morganella morganii* and *Citrobacter freundii*. Ninety percent of *Serratia marcescens* strains were inhibited by 6.25 μg of Sch 34343 per ml. The antibacterial activity of Sch 34343 against *Pseudomonas cepacia* was similar to that of imipenem. The antibacterial activity of Sch 34343 against *Neisseria gonorrhoeae* was greater than that of imipenem. *B. fragilis* was highly susceptible to Sch 34343. The MICs of Sch 34343 were not affected by the inoculum sizes from 10^6 to 10^2 CFU per spot (Table 2).

β-Lactamase stability. We examined the antibacterial activity against β-lactamase-producing strains of various species of bacteria. Sch 34343 showed high antibacterial activity against the strains of bacteria listed in Table 3 except for *Pseudomonas aeruginosa* and *Pseudomonas maltophilia*.

Sch 34343 was found to be quite stable to both penicillin

TABLE 3. Antibacterial activity of Sch 34343 against β-lactamase-producing strains

Strain (β-lactamase)	Type of β-lactamase[a]	MIC (μg/ml) of:		
		Sch 34343	Imipenem	CAZ
Escherichia coli W3630 (Rms212)	Type I PCase	1.6	0.4	0.1
Escherichia coli W3630 (Rms213)	Type II PCase	0.8	1.6	0.1
Escherichia coli ML 1410 (Rte16)	Type III PCase	0.8	0.8	0.1
Escherichia coli C (Rms149)	Type IV PCase	0.8	1.6	0.1
Escherichia coli ML4901 (TEM-1)	PCase	0.4	0.2	0.1
Escherichia coli ML4901 (TEM-2)	PCase	0.4	0.2	0.2
Escherichia coli ML4901 (OXA-1)	PCase	0.4	0.2	0.1
Escherichia coli ML4905 (OXA-2)	PCase	0.4	0.4	3.13
Escherichia coli ML4901 (PSE-1)	PCase	0.4	0.2	0.2
Escherichia coli ML4901 (PSE-3)	PCase	0.4	0.4	0.2
Escherichia coli J53-2 (SHV-1)	PCase	0.4	0.2	0.2
Providencia rettgeri GN5284	CSase	0.8	0.4	0.05
Morganella morganii GN5407	CSase	1.6	3.13	0.05
Proteus vulgaris GN4413	CSase	0.4	0.4	0.1
Escherichia coli GN5482	CSase	0.8	0.1	0.8
Enterobacter cloacae GN7471	CSase	0.4	0.1	1.56
Pseudomonas aeruginosa GN918	CSase	>100	1.6	3.13
Serratia marcescens L-65	CSase	3.13	0.4	0.1
Pseudomonas maltophilia GN12873	CXase	>100	>100	100
Pseudomonas cepacia GN11164	CXase	>100	6.25	0.8
Klebsiella oxytoca GN10650	CXase	0.8	0.2	0.05
Proteus vulgaris GN7919	CXase	0.1	0.05	0.8

[a] See reference 10 for an explanation of types.

β-lactamase (PCase) and various cephalosporin β-lactamases (CSases) (Table 4). Recently, Mitsuhashi et al. proposed that oxyiminocephalosporin β-lactamases (CXases) were classified into two subgroups, type 1 CXases and type 2 CXases. The type 1 CXase is able to hydrolyze monobactams but does not hydrolyze penems and carbapenems, whereas the type 2 CXase is able to hydrolyze penems and carbapenems but does not hydrolyze monobactams (12).

Sch 34343 was also hydrolyzed by type 2 CXases such as those of *Pseudomonas maltophilia* (L-1 enzyme) and *Flavobacterium odoratum* GN14053 (18) but was not hydrolyzed

TABLE 4. Stability of Sch 34343 to various β-lactamases

Organism (β-lactamase)	Type of β-lactamase	K_m (μM)	V_{max} (relative)[a]	K_i (μM)[b]	MIC (μg/ml) of Sch 34343
Escherichia coli W3630 (Rms212)	PCase I	—[c]	—	86.1	1.6
Escherichia coli W3630 (Rms213)	PCase II	—	—	0.15	0.8
Providencia rettgeri GN4430	CSase	—	—	3.54	0.4
Morganella morganii GN5407	CSase	—	—	4.18	1.6
Pseudomonas aeruginosa GN10362	CSase	—	—	1.26	>100
Enterobacter cloacae GN7471	CSase	—	—	0.18	0.4
Proteus vulgaris GN7919	CXase I	—	—	4.64	0.1
Klebsiella oxytoca GN10650	CXase I	—	—	>100	0.8
Pseudomonas cepacia GN11164	CXase I	—	—	21.2	>100
Pseudomonas maltophilia GN12873 (L-2)	CXase I	—	—	7.71	>100
Pseudomonas maltophilia GN12873 (L-1)	CXase II	209	15.3	—	>100
Flavobacterium odoratum GN14053	CXase II	98	3.5	—	NT[d]

[a] Relative rates of hydrolysis of substrates are expressed as the percentage of PCG hydrolysis for PCases and as the percentage of CER hydrolysis for CSases and CXases.

[b] K_i values were determined with CER as the substrate.

[c] —, Not detected.

[d] NT, Not tested.

TABLE 5. Hydrolysis of various β-lactam antibiotics by the purified renal dehydropeptidase 1 from swine kidney

Substrate	K_m (mM)	V_{max} (relative)[a]	K_i (μM)[b]
Glycyldehydrophenylalanine	1.10	100	—[c]
Sch 34343	0.15	0.32	—
Sch 29482	1.00	0.60	—
Imipenem	1.38	1.88	—
Carpetimycin A	0.30	6.95	—
Carpetimycin B	0.32	4.34	—
Aztreonam	—	—	75.4

[a] Relative rate of hydrolysis is expressed as the percentage of glycyldehydrophenylalanine hydrolysis.
[b] K_i values were determined with glycyldehydrophenylalanine as the substrate.
[c] —, Not detected.

by type 1 CXases such as those of *Pseudomonas maltophilia* (L-2 enzyme), *Pseudomonas cepacia*, *K. oxytoca*, and *Proteus vulgaris*.

Hydrolysis of Sch 34343 by renal dehydropeptidase 1. It is known that both carbapenems and penems was inactivated by renal dehydropeptidase 1 (8). The kinetic parameters of this enzyme were determined by Lineweaver-Burk plots. Sch 34343 and Sch 29482 were slightly hydrolyzed by renal dehydropeptidase 1 (Table 5). However, the relative rate of hydrolysis of Sch 34343 was slower than those of imipenem and carpetimycins.

DISCUSSION

Many broad-spectrum antibiotics have been used clinically for the treatment of bacterial infections. However, the isolation frequency of resistant organisms is increasing, perhaps as a result of the increased use of these drugs. Since the discovery of penicillin and cephalosporin C, many derivatives of penems and cephems have been developed that show high antibacterial activity and resistance to various types of β-lactamase.

The present in vitro study was performed to evaluate the antibacterial activity, β-lactamase stability, and renal dehydropeptidase 1 stability of a new β-lactam antibiotic, Sch 34343. We found that Sch 34343 had a broad spectrum of antibacterial activity against both gram-positive and gram-negative bacteria with the exception of *Pseudomonas aeruginosa* and *Pseudomonas maltophilia*. Sch 34343 is more potent than ceftazidime against gram-positive bacteria and has a potency similar to that of ceftazidime against most strains of *Enterobacteriaceae*. Sch 34343 is more active than ceftazidime or imipenem against *B. fragilis*.

Sch 34343 was quite stable to both PCase and CSase, although the previously tested compound Sch 29482 was slightly hydrolyzed by type 1 PCase (11). Sch 34343 as well as imipenem was slightly hydrolyzed only by CXase type 2. Sch 34343 appears to be more stable to renal dehydropeptidase 1 than do other penems and carbapenems because of the low V_{max}. However, the low K_m value of Sch 34343 seems to be disadvantageous, because the affinity of Sch 34343 for renal dehydropeptidase 1 is greater than those of Sch 29482 and imipenem. In summary, Sch 34343 would be an effective agent in the therapy of infections caused by gram-positive and gram-negative bacteria, particularly *Streptococcus faecalis*, *N. gonorrhoeae*, and *B. fragilis*, except for *Pseudomonas aeruginosa* and *Pseudomonas maltophilia*.

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