L-656,575 (OCP-9-176): A NOVEL OXACEPHEM *IN VITRO* ACTIVITY AGAINST AEROBIC AND ANAEROBIC CLINICAL BACTERIAL ISOLATES†

BARBARA WEISSBERGER, GEORGE K. ABRUZZO, ROBERT A. FROMTLING*, MARY E. VALIANT, DANIEL L. SHUNGU and HANS H. GADEBUSCH

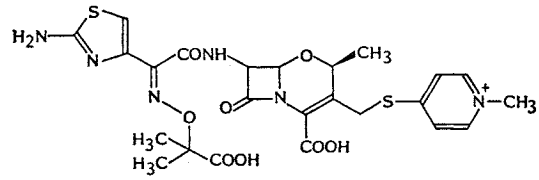
Department of Basic Microbiology, Merck Sharp & Dohme Research Laboratories, P.O. Box 2000 (R 80T 100), Rahway, New Jersey 07065-0900, U.S.A.

(Received for publication January 26, 1988)

L-656,575 (OCP-9-176) is a novel oxacephem superior to ceftazidime in *in vitro* activity against clinical isolates of *Enterobacter* species, methicillin-susceptible *Staphylococcus aureus* and *Staphylococcus epidermidis*, and multiply-resistant *Pseudomonas aeruginosa*. Our results suggest that L-656,575 has a high affinity for penicillin binding proteins of *Pseudomonas* and may bind preferentially to PBP-3 in this organism. L-656,575 is active against β-lactamase derepressed Enterobacteriaceae and ceftazidime-resistant *P. aeruginosa*.

L-656,575 (OCP-9-176; Fig. 1) is a newly discovered oxacephem[1,2] that has a long half-life in mice (18 minutes) and rhesus monkeys (63 minutes)[3,4]. The compound also has been shown to be effective in the treatment of a variety of experimental Gram-negative bacteremias in mice[3,4]. In the present study, the *in vitro* spectrum and potency of L-656,575 in comparison with several other antibiotics against clinical isolates of aerobic and anaerobic bacteria were determined.

Fig. 1. Structure of L-656,575 (OCP-9-176).



Materials and Methods

Antibiotics

L-656,575 was supplied by Meiji Seika Kaisha, Ltd., Yokohama, Japan. Samples of the following antibiotics were provided by the company developing or marketing the drug: Ceftazidime (Glaxo), cefpirome (HR 810; Hoechst-Roussel), ceftriaxone (F. Hoffmann-La Roche Inc.), moxalactam (Eli Lilly and Company), imipenem (Merck & Co.), cefoperazone (Pfizer International Inc.), cefepime (BMY-28142; Bristol-Myers Co.), cefsulodin (Abbott Laboratories), aztreonam (E. R. Squibb & Sons), and piperacillin (Lederle, Ltd.).

Cultures

Cultures used in this evaluation were all clinical isolates. Each was maintained in the Merck Culture Collection at $-70°C$ on Trypticase Soy agar (TSA; BBL) slants or in 15% skim milk. Each isolate was subcultured from the collection and checked for purity prior to use.

MIC: Agar Dilution

The MICs were determined using an agar dilution method. Each compound was prepared ac-

† Presented in part at the 27th Intersci. Conf. on Antimicrob. Agents Chemother., New York, Oct. 4~7, 1987.

Table 1. *In vitro* antibacterial activity[a] of L-656,575 (OCP-9-176) compared to ceftazidime against clinical aerobic bacterial isolates.

Organism (No. tested)	MIC (μg/ml)							
	L-656,575				Ceftazidime			
	Range	MIC_{50}	MIC_{90}	G-MIC	Range	MIC_{50}	MIC_{90}	G-MIC
Acinetobacter sp. (6)	8.0～>128.0	—	128.0	≥71.9	1.0～64.0	—	16.0	8.98
Enterobacter aerogenes (10)	0.06～2.0	0.25	1.0	0.38	0.03～64.0	0.5	32.0	1.41
E. cloacae (10)	0.06～8.0	0.25	4.0	0.66	0.25～128.0	1.0	128.0	2.64
Escherichia coli (20)	0.06～0.5	0.125	0.25	0.16	0.125～0.5	0.25	0.25	0.19
Haemophilus influenzae (20)	≤0.008～1.0	0.125	0.5	≤0.11	≤0.007～1.0	0.06	0.25	0.06
Klebsiella pneumoniae (25)	0.06～0.5	0.25	0.5	0.22	0.125～0.5	0.25	0.5	0.29
Proteus mirabilis (20)	≤0.008～0.5	0.06	0.5	≤0.07	0.03～0.5	0.06	0.5	0.11
P. vulgaris (20)	0.03～2.0	0.06	0.5	0.09	0.03～0.125	0.06	0.125	0.07
Providencia rettgeri (10)	≤0.008～0.5	0.06	0.5	≤0.07	0.016～0.5	0.125	0.25	0.09
P. stuartii (10)	0.06～1.0	0.25	0.5	0.29	0.125～0.5	0.25	0.5	0.29
Pseudomonas aeruginosa (44)	1.0～64.0	8.0	16.0	7.77	0.5～>128.0	4.0	64.0	≥4.68
Pseudomonas sp. (9)	4.0～>128.0	—	128.0	≥17.30	1.0～128.0	—	8.0	3.70
Staphylococcus aureus (16) methicillin-susceptible	2.0～8.0	4.0	4.0	3.08	4.0～16.0	8.0	16.0	8.72
S. epidermidis (15) methicillin-susceptible	0.5～4.0	1.0	2.0	1.26	2.0～16.0	4.0	8.0	4.60
Streptococcus pneumoniae (11)	0.06～32.0	0.25	0.25	0.26	0.03～64.0	0.125	0.25	0.14
S. pyogenes (12)	0.02～1.0	0.5	0.5	0.28	0.02～0.125	0.03	0.06	0.04

[a] Determined by agar dilution assay using multipoint inoculator, inoculum: 10^5 cfu/spot, Trypticase Soy agar, or Trypticase Soy agar supplemented with 5% lysed horse blood and 25 μg/ml NAD for fastidious organisms.

MIC: Lowest concentration showing no visible growth or fewer than 5 discrete colonies.

MIC_{50}: Concentration at which 50% of the strains were inhibited.

MIC_{90}: Concentration at which 90% of the strains were inhibited.

G-MIC: Geometric mean of the MICs.

—: Not determined.

cording to the manufacturer's instructions. Two-fold dilutions were made in appropriate media. One-ml of each antibiotic dilution was mixed with 9 ml of cooled, molten agar in 15×100 mm petri plates to yield final concentrations of drug ranging from 0.008 to 128 μg/ml. For the aerobes, TSA was used; for fastidious cultures, TSA supplemented with 5% lysed horse blood and 25 μg/ml nicotinamide adenine dinucleotide was used. For the anaerobes, brain heart infusion (BHI; Difco) agar supplemented with 5 μg/ml hemin and 0.5 μg/ml menadione was used. Test plates were inoculated using a Denley Multipoint Inoculator (Sussex, England) designed to deliver 1.0 μl aliquots directly onto the agar surface. A final inoculum of 10^4, 10^5 or 10^6 cfu/spot was used for the aerobes, and 10^6 cfu/spot for the anaerobes. The aerobes were incubated at 35°C for 18~20 hours, and the anaerobes were incubated at 35°C for 40 hours in a Forma anaerobic chamber (Marietta, Ohio) under an atmosphere of 10% hydrogen, 8% carbon dioxide and 82% nitrogen. The MIC was defined as the lowest concentration of drug showing no distinct growth or less than five discrete colonies/spot.

MICs and Minimum Bactericidal Concentrations (MBC's): Broth Dilution

MICs and MBC's for L-656,575 and ceftazidime against eight isolates of multiply-resistant *Pseudomonas aeruginosa* were determined using a broth microdilution assay using Dynatech's MIC-2000 system (Chantilly, Virginia). The drugs were prepared as described above, except that Trypticase Soy broth (TSB; BBL) was used, and final drug concentrations in broth ranged from 0.125 to 128 μg/ml. Overnight cultures of the test strains were diluted with TSB and 1.5 μl was added to 50 μl of drug dilution in the test wells. This resulted in an inoculum of approximately 1×10^5 cfu/ml. After incubation at 35°C for 20 hours, the test wells were examined for growth. The MIC was recorded as the lowest concentration of drug where no growth was observed.

From each well of these assay plates, 1.5 μl was transferred to the surface of drug-free TSA using the MIC 2000 system. The agar plates were incubated at 35°C for 20 hours. The lowest drug concentration where no growth or $\leq$5 discrete colonies persisted was recorded as the MBC, indicating that at least 96.7% of the initial inoculum (1×10^5 cfu/ml) had been killed.

Results and Discussion

The *in vitro* antibacterial activity of L-656,575 as compared to that of ceftazidime against a variety of Gram-negative and Gram-positive aerobic clinical isolates is summarized in Table 1. Overall,

Fig. 2. Cumulative percent of susceptible clinical isolates of *Enterobacter* species (20 strains) to L-656,575 (○) and ceftazidime (●).

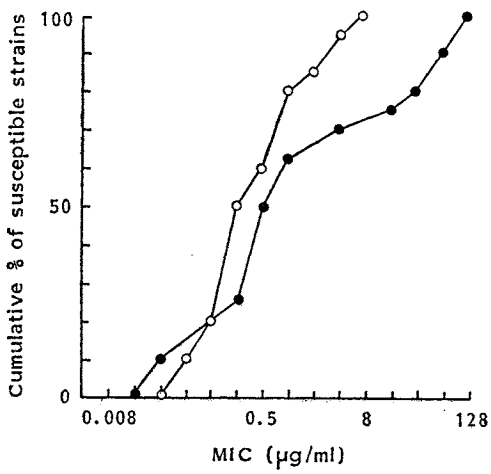


Fig. 3. Cumulative percent of susceptible clinical isolates of methicillin-susceptible *Staphylococcus* species (31 strains) to L-656,575 (○) and ceftazidime (●).

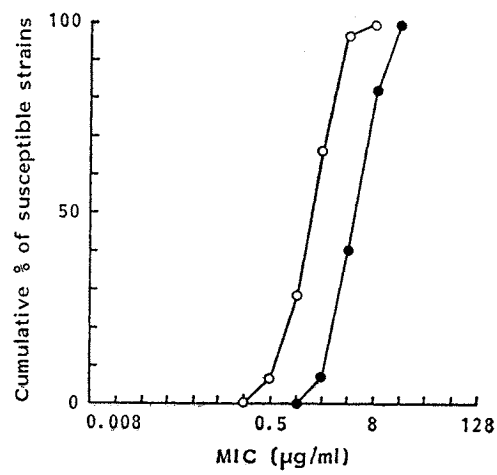


Table 2. *In vitro* activity of L-656,575 and other cephalosporins against selected anaerobic clinical isolates.

Organism	MIC (μg/ml)[a]				
	L-656,575	CPO	CAZ	CRO	MOX
Eubacterium lentum CL102	>128.0	32.0	>128.0	128.0	>128.0
Peptococcus asaccharolyticus CL97	0.5	0.25	0.125	0.03	0.125
P. prevotii CL116	2.0	0.125	0.5	0.25	1.0
Peptostreptococcus anaerobius CL230	8.0	0.125	8.0	0.125	64.0
Propionibacterium acnes CL312	2.0	0.06	0.5	≤0.008	0.5
Clostridium difficile CL51	64.0	128.0	128.0	32.0	>128.0
C. difficile CL183	>128.0	32.0	>128.0	32.0	>128.0
C. difficile CL222	32.0	32.0	32.0	32.0	128.0
C. perfringens CL62	8.0	1.0	4.0	2.0	0.125
C. perfringens CL153	8.0	1.0	4.0	8.0	0.25
C. perfringens CL256	16.0	1.0	4.0	16.0	2.0
Bacteroides fragilis CL16	>128.0	>128.0	>128.0	>128.0	16.0
B. fragilis CL30	4.0	4.0	4.0	4.0	0.125
B. fragilis CL73	16.0	8.0	4.0	1.0	0.125
B. distasonis CL71	>128.0	>128.0	>128.0	>128.0	4.0
B. distasonis CL180	2.0	0.5	1.0	0.03	0.25
B. ovatus CL236	>128.0	>128.0	>128.0	>128.0	>128.0
B. thetaiotaomicron CL158	128.0	64.0	128.0	32.0	0.125
B. thetaiotaomicron CL235	>128.0	>128.0	>128.0	128.0	4.0
B. vulgatus CL88	32.0	64.0	32.0	2.0	1.0
B. vulgatus CL243	16.0	32.0	16.0	1.0	0.25

[a] Agar dilution assay, multipoint inoculator, brain heart infusion agar supplemented with 5 μg/ml hemin and 0.5 μg/ml menadione, inoculum: 10^6 cfu/spot, incubation of 35°C for 40 hours. MIC: Lowest concentration showing no visible growth or fewer than 5 discrete colonies.

CPO: Cefpirome, CAZ: ceftazidime, CRO: ceftriaxone, MOX: moxalactam.

L-656,575 demonstrated a broad spectrum of antibacterial activity with excellent potency comparable to ceftazidime. The potency of L-656,575 was superior to that of ceftazidime against isolates of *Enterobacter* species (Fig. 2, Table 1), methicillin-susceptible *Staphylococcus aureus* and *Staphylococcus epidermidis* (Fig. 3, Table 1), and *P. aeruginosa* (Table 1). The potency of L-656,575 was less than that observed with ceftazidime against isolates of *Acinetobacter* species, *Pseudomonas* species other than *P. aeruginosa*, and *Streptococcus pyogenes*. Both drugs were comparable in potency against the other clinical isolates tested (Table 1).

The *in vitro* antibacterial activity of L-656,575 and other cephalosporins against selected anaerobic isolates is summarized in Table 2. Moxalactam was the most active compound, followed in relative activity by ceftriaxone, cefpirome, ceftazidime and L-656,575. L-656,575 demonstrated good activity against several of the anaerobic isolates, but relative potency was the lowest when the drug was compared to the other four cephalosporins tested.

The comparative activities of L-656,575 and selected β-lactam antibiotics against fifteen multiply-resistant strains of *P. aeruginosa* are summarized in Table 3. The data show that L-656,575 and imipenem were the most active compounds. At drug concentrations of ≤16 μg/ml, only imipenem had better activity than L-656,575 against these multiply-resistant isolates. Also of significant importance was the fact that L-656,575 was not cross-resistant with the other β-lactam antibiotics tested.

When the MBC/MIC ratios for L-656,575, imipenem, and ceftazidime against eight isolates of multiply-resistant *P. aeruginosa* were compared, the L-656,575 ratio was never more than 1.0, whereas

Table 3. Comparative activity of L-656,575 and selected β-lactams against multiply-resistant strains of *Pseudomonas aeruginosa*.

Strain No.	MIC (μg/ml)[a]										Resistant to[b]
	L-656,575	ATM	PIP	CAZ	CEF	CPO	IPM	CFS	CFP	MOX	
CL725	16.0	16.0	128.0	4.0	128.0	128.0	2.0	8.0	16.0	32.0	AN, CBPC, GM, NN, IPM, PIP
CL2014	16.0	16.0	>128.0	4.0	64.0	64.0	4.0	16.0	128.0	32.0	AN, CBPC, GM, NN, PIP
CL2448	16.0	64.0	>128.0	32.0	32.0	128.0	16.0	32.0	>128.0	>128.0	AN, CBPC, GM, PIP, IPM
CL2450	16.0	64.0	>128.0	128.0	64.0	128.0	2.0	64.0	>128.0	>128.0	CBPC, CAZ, PIP
CL2533	64.0	64.0	32.0	8.0	8.0	8.0	16.0	16.0	64.0	>128.0	AN, CBPC, GM, IPM
CL2720	8.0	16.0	16.0	4.0	4.0	8.0	16.0	4.0	16.0	64.0	IPM
CL2820	8.0	8.0	8.0	2.0	2.0	4.0	16.0	4.0	16.0	32.0	IPM
CL2850	16.0	16.0	>128.0	4.0	32.0	16.0	32.0	128.0	>128.0	64.0	AN, CBPC, GM, IPM, PIP
CL2860	8.0	128.0	>128.0	>128.0	32.0	64.0	16.0	32.0	>128.0	>128.0	AN, CBPC, GM, CAZ, IPM, PIP
CL2950	8.0	16.0	8.0	1.0	4.0	8.0	16.0	2.0	8.0	16.0	IPM, GM
CL2979	32.0	128.0	>128.0	64.0	32.0	128.0	64.0	64.0	>128.0	>128.0	AN, CBPC, GM, IPM, CAZ
CL2997	16.0	8.0	8.0	8.0	8.0	16.0	32.0	4.0	16.0	32.0	AN, GM, MOX, IPM, PIP
CL3015	8.0	16.0	128.0	16.0	16.0	32.0	32.0	8.0	128.0	128.0	AN, CBPC, GM, MOX, IPM, PIP
CL3020	32.0	64.0	>128.0	64.0	32.0	64.0	8.0	32.0	>128.0	>128.0	CBPC, CAZ, MOX, PIP
CL3092	8.0	64.0	>128.0	128.0	32.0	128.0	32.0	32.0	>128.0	>128.0	IPM, MOX, CAZ, CBPC, PIP
G-MIC[c]	14.52	30.06	>58.52	>13.9	19.32	35.16	13.91	16.02	>58.35	>70.20	

[a] Agar dilution assay, multipoint inoculator, Trypticase Soy agar, 10^5 cfu inoculum. MIC: Lowest concentration showing no visible growth or fewer than 5 discrete colonies.

[b] Resistance as determined from NCCLS approved standard M2-A3, Vol. 4, 1986.

[c] G-MIC: Geometric mean of the MICs.

ATM: Aztreonam, PIP: piperacillin, IPM: imipenem, CEF: cefepime, CFS: cefsulodin, AN: amikacin, CBPC: carbenicillin, CAZ: ceftazidime, GM: gentamicin, NN: tobramycin, MOX: moxalactam, CPO: cefpirome, CFP: cefoperazone.

Table 4. Bactericida lactivity of L-656,575, imipenem and ceftazidime for multiply-resistant clinical strains of *Pseudomonas aeruginosa*.

Strain No.	Resistant to[c]	MIC (μg/ml)[a]			MBC (μg/ml)[b]			Ratio MBC/MIC		
		L-656,575	IPM	CAZ	L-656,575	IPM	CAZ	L-656,575	IPM	CAZ
CL1440	GM	4.0	4.0	1.0	4.0	8.0	1.0	1	2	1
CL1999	AN, CB, GM	8.0	4.0	2.0	8.0	4.0	2.0	1	1	1
CL2049	GM, NN	4.0	2.0	1.0	4.0	2.0	2.0	1	1	2
CL2411	AN, GM	8.0	1.0	2.0	8.0	4.0	2.0	1	4	1
CL2850	AN, CB, GM, IPM, PIP	16.0	16.0	4.0	16.0	32.0	4.0	1	2	1
CL2979	AN, CB, GAZ, GM, IPM	16.0	32.0	32.0	16.0	32.0	32.0	1	1	1
GL3015	AN, CB, GM, IPM, MOX, PIP	8.0	16.0	16.0	8.0	32.0	16.0	1	2	2
CL3092	CB, CAZ, IPM, MOX, PIP	8.0	16.0	64.0	8.0	16.0	128.0	1	1	2

[a] Lowest concentration in Trypticase Soy broth with no visible growth after 20 hours incubation at 35°C.
[b] Lowest concentration at which 96.7% of initial inoculum (1×10^5 cfu/ml) was killed.
[c] Resistance determined by standard NCCLS agar dilution or agar diffusion disk methods.
AN: Amikacin, CAZ: ceftazidime, CB: carbenicillin, GM: gentamicin, IPM: imipenem, MOX: moxalactam, NN: tobramycin, PIP: piperacillin.

Table 5. *In vitro* activity of L-656,575 vs. laboratory strains known to possess either a plasmid or a chromosomally mediated β-lactamase.

Organism	MIC (μg/ml)[a]	
	10^4 cfu/spot	10^8 cfu/spot
Escherichia coli TEM 2^+	0.03	0.125
Enterobacter cloacae P99$^+$	0.5	2.0
Klebsiella oxytoca K1$^+$	2.0	32.0
Pseudomonas aeruginosa RPL11$^+$	2.0	4.0

[a] Agar dilution method, multipronged inoculator, Trypticase Soy agar, incubation at 35°C for 20 hours.

those for both imipenem and ceftazidime exceeded 1.0 against four and three of these isolates, respectively (Table 4). Microscopic examinations of broth cultures of *P. aeruginosa* at subinhibitory concentrations of the compound revealed the predominance of long chains of elongated cells (not shown). These results suggest that L-656,575 has a high affinity for penicillin binding proteins of *Pseudomonas*, and that it may bind preferentially to PBP-3 in this organism.

In a separate study using an agar dilution method, potent *in vitro* activities were demonstrated for L-656,575 against laboratory isolates of *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella oxytoca* and *P. aeruginosa* known to possess either plasmid or chromosomally-mediated β-lactamases, even at an inoculum concentration of 10^6 cfu/spot. These data suggest that the compound is very resistant to hydrolysis by bacterial β-lactamases (Table 5).

In conclusion, L-656,575 is a novel 2-methyloxycephalosporin with *in vitro* antibacterial activity superior to ceftazidime against *Enterobacter* species, methicillin-susceptible *S. aureus* and *S. epidermidis*, and multiply-resistant *P. aeruginosa*. This compound is active against β-lactamase derepressed Enterobacteriaceae and ceftazidime-resistant *P. aeruginosa*.

References

1) SHIBAHARA, S.; T. OKONOGI, Y. MURAI, T. YOSHIDA, S. KONDO & B. G. CHRISTENSEN: Synthesis and *in vitro* antibacterial activity of OCP-9-176, a potent 2-methyloxacephalosporin (2MO). Program and

Abstracts of the 27th Intersci. Conf. on Antimicrob. Agents Chemother., No. 647, p. 209, New York, Oct. 4~7, 1987

2) SHIBAHARA, S.; T. OKONOGI, Y. MURAI, T. KUDO, T. YOSHIDA, S. KONDO & B. G. CHRISTENSEN: Synthesis of a novel 2β-methyl-1-oxacephalosporin, OCP-9-176. J. Antibiotics 41: 1154~1157, 1988

3) GILFILLAN, E.; B. PELAK, J. BLAND, S. HADLEY & H. GADEBUSCH: Pharmacokinetics and experimental chemotherapy of L-656,575 (OCP-9-176). Program and Abstracts of the 27th Intersci. Conf. on Antimicrob. Agents Chemother., No. 649, p. 210, New York, Oct. 4~7, 1987

4) GILFILLAN, E. C.; B. A. PELAK, R. A. FROMTLING, J. BLAND, S. HADLEY & H. H. GADEBUSCH: L-656,575 (OCP-9-176): A novel oxacephem. Pharmacokinetics and experimental chemotherapy. J. Antibiotics 41: 1137~1141, 1988