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In Vitro Studies of l-Oxacephalosporin (LY 127935), a New Beta-Lactam Antibiotic

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LY 127935 exhibited potent, broad-spectrum activity in vitro against 573 clinical isolates of bacteria. At concentrations below 6.25 μg/ml, it inhibited the majority of isolates of all organisms except *Pseudomonas aeruginosa*, and was active even against organisms which usually exhibit resistance such as *Serratia marcescens* and *Enterobacter* spp. A rise in inoculum from 10^5 to 10^7 cells per ml significantly reduced the activity of this drug. LY 127935 was more active than cephalothin, cefamandole, or cefoxitin against the *Enterobacteriaceae*.

Gram-negative bacillary infections are a major cause of morbidity and mortality among hospitalized patients. The search for new antibiotics and derivatives with broad-spectrum activity against major pathogens is of great importance, especially since the widespread use of currently available antibiotics at times has been associated with the emergence of resistant isolates. LY 127935, $C_{20}H_{18}N_6O_9SNa_2$, is a new, semisynthetic beta-lactam antibiotic which is the first of a new class of β-lactam antibiotics reported to have exceptional biological properties, including activity against multiply-resistant pathogens and aminoglycoside-resistant strains (2). Because of its broad-spectrum activity against most organisms causing infection in the compromised host, we determined the activity of LY 127935 against organisms isolated from cancer patients hospitalized at this institution and compared its activity with that of cefamandole, cefoxitin, cephalothin, tobramycin, carbenicillin, mezlocillin, and piperacillin.

Susceptibility tests were performed simultaneously in duplicate on 425 clinical isolates of gram-negative bacilli and 148 clinical isolates of gram-positive cocci by a microbroth dilution method in minimum inhibitory concentration (MIC) plates (Cook Laboratory Products, Division of Dynatech Laboratories, Inc.). Organisms included 100 isolates of *Escherichia coli*, 75 isolates of *Enterobacter* spp., 100 isolates of *Klebsiella pneumoniae*, 28 isolates of *Proteus mirabilis*, 15 isolates of indole-positive *Proteus* spp., 117 isolates of *P. aeruginosa*, 32 isolates of *S. marcescens*, 45 isolates of *Streptococcus pyogenes*, 16 isolates of *Streptococcus pneumoniae*, 37 isolates of *Staphylococcus aureus* resistant to penicillin G, and 47 isolates of *S. aureus* susceptible to penicillin G. Materials and methods were the same as in previous studies (5) except that plates were prepared and inoculated by a Dynatech MIC-2000 System (Cooke Laboratory Products, Division Dynatech Laboratories, Inc.). Plates were frozen at −35°C and thawed before inoculation. *Enterobacter cloacae* ATCC 13047, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 27736, *Proteus vulgaris* ATCC 6380, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 25923 were included as control organisms.

LY 127935, cefamandole, cephalothin, and tobramycin were supplied by Eli Lilly and Co., Indianapolis, Ind. Cefoxitin was supplied by Merck, Sharp and Dohme Research Laboratories, Rahway, N.J. Mezlocillin was supplied by Delbay Research Corp., Florham, N.J. Piperacillin was supplied by Lederle Laboratories, Pearl River, N.Y. Carbenicillin was supplied by Beecham Laboratories, Bristol, Tenn.

The activity of LY 127935 against gram-negative bacilli and gram-positive cocci is summarized in Table 1. A concentration of 6.25 μg/ml inhibited the majority of isolates of all organisms except *P. aeruginosa*. A concentration of 25 μg/ml inhibited only 47% of these isolates.

The concentration of LY 127935 required for bactericidal activity against 100% of isolates was the same as the MIC for *Enterobacter* spp. and indole-positive *Proteus* spp. A twofold-higher concentration than the MIC was required for bactericidal activity against *E. coli*, and a fourfold-higher concentration than the MIC for *K. pneumoniae* and *S. marcescens* (Table 1). A substantial difference was observed between the MICs and minimum bactericidal concentrations (MBCs) for *P. mirabilis* and *P. aeruginosa*. LY 127935 was inhibitory against 100% of isolates of *P. mirabilis* at a concentration of 1.56 μg/ml, but it was bactericidal against only 50% at this concentration. It was inhibitory against 67% of isolates of *P. aeruginosa* at a concentration of 50 μg/ml, but it was bactericidal against only 27% at this same concentration.

The effect of inoculum size on the activity of LY 127935 against 10 isolates each of *E. coli*, *K.*

TABLE 1. *In vitro activity of LY 127935*

Organism	No. of isolates tested	Activity tested	MIC or MBC (μg/ml) which inhibited % of isolates:		
			50	75	90
Enterobacter spp.	75	MIC	0.39	6.25	25
		MBC	3.12	12.5	25
E. coli	100	MIC	0.20	0.39	0.39
		MBC	0.20	0.39	0.78
K. pneumoniae	100	MIC	0.20	0.39	0.39
		MBC	0.20	0.39	1.56
P. mirabilis	28	MIC	0.78	0.78	0.78
		MBC	1.56	6.25	6.25
Proteus spp. (indole positive)	15	MIC	0.10	0.39	6.25
		MBC	0.20	0.78	6.25
P. aeruginosa	117	MIC	50	100	>200
		MBC	>200	>200	>200
S. marcescens	32	MIC	0.39	0.78	1.56
		MBC	0.78	3.12	6.25
S. aureus (penicillin G susceptible)	47	MIC	6.25	6.25	6.25
		MBC	6.25	6.25	6.25
S. aureus (penicillin G resistant)	37	MIC	6.25	6.25	12.5
		MBC	6.25	12.5	12.5
S. pyogenes	45	MIC	0.78	0.78	1.56
		MBC	0.78	>3.12	>3.12
S. pneumoniae	16	MIC	1.56	1.56	3.12
		MBC	1.56	1.56	3.12

pneumoniae, and *P. aeruginosa* is shown in Table 2. An increase in inoculum from 10^5 to 10^7 cells per ml significantly reduced the activity of this drug.

The activity of LY 127935 in Mueller-Hinton (MH) media at pH 6.4, 7.2, and 8.0 against 30 isolates of gram-negative bacilli was determined. The concentration of LY 127935 required to inhibit all 10 isolates of *E. coli* was twofold higher at pH 8.0 than at pH 6.4 or 7.2. The concentration required to inhibit all 10 isolates of *K. pneumoniae* was twofold higher at pH 8.0 and 7.2 than at pH 6.4. The concentration required to inhibit all 10 isolates of *P. aeruginosa* was the same at pH 6.4, 7.2, and 8.0.

The activity of LY 127935 against 30 isolates of gram-negative bacilli in MH broth, Trypticase soy broth (TSB, BBL Microbiology Systems), brain heart infusion broth (BHI), and nutrient broth (NB) was determined. The concentrations of LY 127935 required to inhibit all 10 isolates of *E. coli* were 0.20 μg/ml in MH and 0.39 μg/ml in TSB, BHI, and NB. The concentrations required to inhibit all 10 isolates of *K. pneumoniae* were 0.39 μg/ml in MH, 0.78 μg/ml in TSB and BHI, and 1.56 μg/ml in NB. The concentrations required to inhibit all 10 isolates of *P. aeruginosa* were 25 μg/ml in TSB and BHI and 50 μg/ml in MH and NB.

The activity of LY 127935 was compared with the activity of cefamandole, cefoxitin, cephalothin, tobramycin, carbenicillin, mezlocillin, and piperacillin against gram-negative bacilli (Table 3). LY 127935 exhibited the greatest potency and spectrum of activity, inhibiting a majority of isolates of all organisms except *P. aeruginosa* at concentrations below 6.25 μg/ml. It was the most active antibiotic against the *Enterobacteriaceae*. Tobramycin was the second most active antibiotic and had the greatest activity against *P. aeruginosa*. The cephalosporins, in general, were quite active against *E. coli*, *K. pneumoniae*, and *P. mirabilis*, although LY 127935 was the most potent, inhibiting a majority of isolates at lower concentrations. The penicillins were also quite active against these organisms, although approximately 30% of isolates of *E. coli* and 86% of isolates of *K. pneumoniae* were resistant to carbenicillin. Approximately 30% of isolates of

TABLE 2. *Effect of inoculum size on activity of LY 127935*

Organism	No. of isolates tested	Inoculum sizes (cells/ml)	MIC (μg/ml) required to inhibit % of isolates:	
			80	100
E. coli	10	10^5	0.20	0.20
		10^7	>200	>200
K. pneumoniae	10	10^5	0.20	1.56
		10^7	>200	>200
P. aeruginosa	10	10^5	100	100
		10^7	>200	>200

TABLE 3. *Comparative in vitro activity of LY 127935 and seven other drugs against 265 isolates of gram-negative bacilli*

Organism (no. of isolates)	Drug[a]	MIC (μg/ml) which inhibited % of isolates:		
		50	75	90
Enterobacter spp. (48)	127935	0.39	6.25	25
	Cefam	25	>200	>200
	Cefox	>200	>200	>200
	Cephal	>200	>200	>200
	Tobra	1.56	3.12	12.5
	Carb	25	>200	>200
	Mezlo	12.5	>200	>200
	Piper	6.25	>200	>200
E. coli (50)	127935	0.20	0.20	0.39
	Cefam	0.78	3.12	12.5
	Cefox	3.12	3.12	12.5
	Cephal	12.5	12.5	100
	Tobra	0.78	1.56	1.56
	Carb	6.25	>50	>50
	Mezlo	1.56	100	>200
	Piper	1.56	25	>200
K. pneumoniae (50)	127935	0.20	0.39	0.39
	Cefam	1.56	6.25	>200
	Cefox	3.12	3.12	6.25
	Cephal	3.12	12.5	100
	Tobra	0.78	0.78	0.78
	Carb	>200	>200	>200
	Mezlo	12.5	>200	>200
	Piper	6.25	>200	>200
P. mirabilis (28)	127935	0.78	0.78	0.78
	Cefam	0.78	1.56	1.56
	Cefox	3.12	3.12	6.25
	Cephal	3.12	6.25	6.25
	Tobra	1.56	3.12	3.12
	Carb	1.56	1.56	1.56
	Mezlo	0.78	0.78	1.56
	Piper	0.39	0.39	0.78
Proteus spp. (indole positive, 15)	127935	0.10	0.39	6.25
	Cefam	25	50	>50
	Cefox	12.5	12.5	12.5
	Cephal	>50	>50	>50
	Tobra	0.78	1.56	3.12
	Carb	1.56	1.56	25
	Mezlo	1.56	3.12	3.12
	Piper	0.78	3.12	3.12
P. aeruginosa (50)	127935	25	100	>200
	Cefam	>200	>200	>200
	Cefox	>200	>200	>200
	Cephal	>200	>200	>200
	Tobra	0.78	1.56	3.12
	Carb	100	>200	>200
	Mezlo	100	>200	>200
	Piper	12.5	50	>200
S. marcescens (32)	127935	0.39	0.78	1.56
	Cefam	200	>200	>200
	Cefox	25	50	50
	Cephal	>200	>200	>200
	Tobra	3.12	6.25	12.5
	Carb	12.5	>200	>200
	Mezlo	6.25	50	>200
	Piper	3.12	25	200

these organisms were also resistant to mezlocillin and piperacillin at concentrations of 200 μg/ml. LY 127935 and cefoxitin were the most active cephalosporins against isolates of indole-positive *Proteus* spp. Cephalothin had no activity against these isolates at a concentration of 50 μg/ml. Carbenicillin was the most active penicillin against these isolates, although mezlocillin and piperacillin were also quite active. LY 127935 was the only cephalosporin with good activity against *Enterobacter* spp. and *S. marcescens*. Its activity was greater than that of tobramycin against these isolates. Forty-six percent of isolates of *Enterobacter* spp. and 34% of isolates of *S. marcescens* were resistant to carbenicillin at a concentration of 200 μg/ml. Thirty-three percent of isolates of *Enterobacter* spp. and approximately 20% of isolates of *S. marcescens* were resistant to mezlocillin and piperacillin at a concentration of 200 μg/ml. LY 127935 was the only cephalosporin with any activity against *P. aeruginosa*, inhibiting 50% of these isolates at a concentration of 25 μg/ml. Tobramycin was the most active antibiotic against these organisms, inhibiting greater than 75% at a concentration of 1.56 μg/ml.

LY 127935 exhibited potent and broad-spectrum activity against gram-positive cocci and gram-negative bacilli, inhibiting a majority of isolates of all organisms except *P. aeruginosa*. The concentrations of LY 127935 required to inhibit the organisms should be readily achievable in humans assuming that the pharmacology of this drug is similar to that of the cephalosporins. The MBCs were significantly greater than the MICs for *P. mirabilis* and *P. aeruginosa*, an observation that may have important clinical implications. Also, inoculum size had a significant effect on the activity of this drug. Hence, whether its potent in vitro activity under standard conditions will be reflected in its clinical activity remains to be determined.

In comparison studies, the activity of LY 127935 was greater than that of tobramycin, except for its activity against *P. aeruginosa*. This greater activity is important because of the nephrotoxic potential of aminoglycoside antibiotics. Furthermore, an increasing number of gram-negative bacilli have developed resistance to these antibiotics in recent years (3). Since the cephalosporins are less toxic antibiotics than the aminoglycosides, higher doses can be administered, providing a higher serum concentration-to-MIC ratio, which may be important in treating serious infections, especially in the compro-

[a] 127935, LY 127935; Cefam, cefamandole; Cefox, cefoxitin; Cephal, cephalothin; Tobra, tobramycin; Carb, carbenicillin; Mezlo, mezlocillin; Piper, piperacillin.

mised host (4). Although LY 127935 has greater inhibitory activity against *P. aeruginosa* than carbenicillin, its bactericidal activity is less; hence, whether it will be as active as carbenicillin against *Pseudomonas* infections remains to be determined.

The spectrum of activity of LY 127935 is similar to that of HR 756 (1). The data of Neu et al. suggest that HR 756 may be somewhat more active than LY 127935. However, it is difficult to compare results from different studies, especially when different organisms are examined. As a general rule, clinical isolates obtained from patients at this institution are more resistant to antibiotics, probably a consequence of the extensive use of antibiotic therapy. A simultaneous comparison between HR 756 and LY 127935 would be of interest.

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