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LY-127935: a Novel Beta-Lactam Antibiotic with Unusual Antibacterial Activity

GARY M. TRAGER,* G. WESLEY WHITE, VICTORIA M. ZIMELIS, AND ANAND P. PANWALKER

University of Illinois Hospital, Section of Infectious Diseases, Department of Medicine, Abraham Lincoln School of Medicine, University of Illinois College of Medicine, Chicago, Illinois 60612

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The in vitro activity of LY-127935, a new beta-lactam antibiotic, was examined by using 370 clinical bacterial strains. In comparison with several other beta-lactam agents, LY-127935 was the most inhibitory against the *Enterobacteriaceae*. It was remarkably active against multi-drug-resistant strains of *Enterobacter* spp., *Serratia* spp., and *Pseudomonas aeruginosa*. LY-127935 had four- to eightfold greater activity than did cefoxitin against *Bacteroides fragilis*. Production of beta-lactamase by *Enterobacteriaceae* did not influence the minimal inhibitory concentration of LY-127935. However, the beta-lactamase-producing strains of *B. fragilis* and *Haemophilus influenzae* had generally higher minimal inhibitory concentrations. LY-127935 was the least active agent tested against gram-positive aerobic cocci. Variations in pH, salt content, protein content, or inocula size had little influence on susceptibility to LY-127935. Although combination studies with LY-127935 and gentamicin demonstrated synergy for *P. aeruginosa*, the rates of killing for the combination and for gentamicin alone were similar.

The recent discovery of several new semisynthetic cephalosporin and cephamycin antibacterial agents has led to the development of LY-127935. This agent has a broader spectrum of activity than the previously available cephalosporins (Compound LY-127935: information for in vitro studies, Lilly Research Laboratories, 1978). Its range of activity includes gram-positive aerobic cocci, *Enterobacteriaceae, Pseudomonas aeruginosa, Haemophilus influenzae,* and *Bacteroides fragilis*. This report compares various in vitro characteristics of LY-127935 with those of other beta-lactam compounds.

MATERIALS AND METHODS

Antibiotics. LY-127935, cephalothin, cefamandole, and penicillin G were supplied by Lilly Research Laboratories, piperacillin was supplied by American Cyanamid Co., Lederle Laboratories Div., and cefoxitin was supplied by Merck Sharp & Dohme. Gentamicin was provided by Schering Laboratories, and ampicillin and methicillin were from Bristol Laboratories.

Susceptibility tests. A total of 370 clinical isolates were tested for antimicrobial susceptibility by using the microdilution technique with Mueller-Hinton broth. The inoculum size was 10^5 to 10^6 organisms per ml for all study strains. The lowest concentration of antibiotic which showed no visible growth after 18 h of incubation at 37°C was defined as the minimal inhibitory concentration (MIC). The minimal bactericidal concentration (MBC) was tested for by inoculating appropriate agar with the entire contents of each well containing antibiotic concentrations equal to or greater than the MIC. The MBC, defined as 99.9% killing of the initial inoculum, was then read as the lowest concentration at which no growth was observed after overnight incubation. *H. influenzae* was tested in Mueller-Hinton broth supplemented with 2% supplement C (Difco Laboratories) and incubated in CO_2 at 37°C. *B. fragilis* was tested by an agar dilution method (5) employing a Steers replicator for inoculation. After 48 h of anaerobic incubation at 37°C, the MIC of each strain was read as the lowest concentration of the drug yielding no growth, a barely visible haze, or one discrete colony.

The effect of various environmental influences on the susceptibilities of selected strains of LY-127935 was examined. This was accomplished by addition of 4% NaCl, adjustment of the pH, or addition of inactivated serum to Mueller-Hinton broth.

Killing rates using multiples of the MIC. An inoculum of 10^6 organisms per ml was exposed to 1, 4, or 16 times the MIC of LY-127935. Samples were removed initially and at various intervals up to 24 h, diluted, and plated on appropriate agar. Killing rates were determined for one strain each of *Escherichia coli, Klebsiella* spp., *Proteus mirabilis,* and *P. aeruginosa*.

Antibiotic combination studies. LY-127935, gentamicin, or both agents were added at four times the MIC to an inoculum of 10^6 organisms per ml of selected strains of enterococci and *P. aeruginosa*. Samples were removed initially and at various intervals up to 24 h and plated on appropriate agar to examine the killing kinetics. Bacterial inhibition by the combination of LY-127935 and gentamicin was studied by the microtiter checkerboard technique, and isobolograms were constructed. The MIC of each antibiotic was

assigned a value of one. When the two agents were tested in combination, synergy was defined as existing when the sum of the fractional MICs was less than one (1).

Beta-lactamase production. The production of beta-lactamase by all of the study strains was qualitatively determined by using Nitrocefin, a chromogenic cephalosporin (3), which was kindly provided by C. H. O'Callaghan, Glaxo-Allenburys Research.

Studies with clavulanic acid. Clavulanic acid (4) was added at a concentration of 1, 5, or 10 μg/ml to Mueller-Hinton broth. Susceptibility studies were done by using this broth alone and in combination with LY-127935 for selected strains of *Staphylococcus aureus* and *P. aeruginosa* as described above. Clavulanic acid was supplied by Glaxo-Allenburys Research.

RESULTS

The comparative inhibitory activity of the various antibiotics studied is shown in Table 1. Against the gram-positive aerobic cocci, LY-127935 did not have a significant advantage over the other agents tested. Cephalothin was the most active agent against *Staphylococcus aureus* and *S. epidermidis*, whereas LY-127935 was the least active (Table 1). Against *Streptococcus pneumoniae*, the activity of penicillin G was far superior to that of LY-127935 and cefoxitin. Thirteen strains of *S. pyogenes* had identical susceptibility patterns to LY-127935 and cefoxitin, whereas penicillin G was significantly more active. Enterococci were uniformly resistant to both LY-127935 and cefoxitin, whereas ampicillin was highly active.

The activity of LY-127935 against *Enterobacteriaceae* was clearly superior to the activities of the other beta-lactam derivatives. This was particularly noteworthy with *E. coli, Klebsiella* spp., and *Proteus* spp. (Table 1). Strains of *Enterobacter* resistant to cefamandole were uniformly susceptible to LY-127935 (MIC ≤ 4.0 μg/ml). This new agent was also active against *Serratia* spp., including 20 multi-drug-resistant strains. More than 90% of the strains of *P. aeruginosa* were inhibited by LY-127935, 32 μg/ml. Although piperacillin was the most effective agent against gentamicin-susceptible strains, LY-127935 was the most active agent (MIC against 90% of the strains [MIC_{90}] = 32 μg/ml) against 19 gentamicin-, piperacillin-, and carbenicillin-resistant isolates.

H. influenzae was more susceptible to LY-127935 than to ampicillin. One beta-lactamase-producing strain that was ampicillin resistant (MIC = 250 μg/ml) was very sensitive to LY-127935 (MIC = 0.12 μg/ml). When compared with cefoxitin, LY-127935 had at least eightfold more activity against *B. fragilis* at MIC_{75}. The 21 strains that were beta-lactamase-negative by the chromogenic cephalosporin method were inhibited by less than 1.0 μg of LY-127935 per ml. However, MICs of LY-127935 against the five isolates of *B. fragilis* that produced beta-lactamase were either 4.0 or 8.0 μg/ml.

The effect of various environmental influences on the inhibition of selected strains of *Enterobacteriaceae* and *P. aeruginosa* by LY-127935 was examined. When the medium was modified by increasing the protein concentration to 50%, or the NaCl content to 4%, no change in susceptibilities was observed. At a pH of 6.0, 7.0, or 8.0, the inhibitory activity of LY-127935 remained constant. However, at pH 5.0, there was a four- to eightfold increase in the MICs of the drug against *Klebsiella* spp. and *Enterobacter* spp. The inhibitory activity of LY-127935 was not altered by varying the inoculum size from 10^2 to 10^6 colony-forming units per ml.

The rate of bacterial killing for four species of gram-negative bacilli by multiples of the MIC of LY-127935 is shown in Fig. 1. For all the organisms, there was increased killing with increasing concentration of the antibiotic. However, at 6 h, the difference in killing activity between 4 and 16 times the MIC was minimal for all organisms except *P. mirabilis*.

Shown in Fig. 2 are the results of two of the combination studies done with LY-127935 and gentamicin for a sensitive strain of *P. aeruginosa* (LY-127935 MIC = 32.0 μg/ml; gentamicin MIC = 1.0 μg/ml). The rate of bacterial killing was evaluated at four times the MIC for each agent alone and in combination. In the presence of LY-127935, the killing rate was slow, as represented by the relatively horizontal curve. The addition of gentamicin to LY-127935 did not significantly increase the rate of killing, when compared with that for gentamicin alone. The isobologram, however, clearly demonstrates synergy. Similar results were obtained with a strain of enterococcus (LY-127935 MIC > 500 μg/ml; gentamicin MIC = 8.0 μg/ml). Although synergy was demonstrated for this strain, the concentration of LY-127935 required was still high (64 μg/ml).

The stability of LY-127935 to beta-lactamase inactivation was investigated by using the chromogenic cephalosporin. With the *Enterobacteriaceae*, the beta-lactamase-producing strains were as sensitive to LY-127935 as were the beta-lactamase-negative isolates. The enzyme-producing strains, however, were relatively resistant to cephalothin and piperacillin. Beta-lactamase production correlated with a four- to eightfold increase in the MIC of LY-127935 against *B. fragilis*.

The effect of the addition of clavulanic acid at various concentrations in reducing the MICs of LY-127935 against 24 strains of *S. aureus* and 19

TABLE 1. *Comparative in vitro activity of LY-127935*

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)			
		Range	50%	75%	90%
S. aureus (22)	LY-127935	8–125	8	8	8
	Methicillin	2–32	4	4	4
	Cephalothin	0.5–1	0.5	0.5	0.5
	Cefoxitin	4–32	4	4	4
S. epidermidis (22)	LY-127935	8–>500	16	32	250
	Methicillin	2–>500	4	16	64
	Cephalothin	<0.25–250	<0.25	1	64
	Cefoxitin	2–>500	4	8	64
S. pneumoniae (15)	LY-127935	0.016–4	1	2	4
	Penicillin G	<0.001–0.07	0.005	0.02	0.04
	Cefoxitin	0.008–1	0.25	1	1
S. pyogenes (13)	LY-127935	<0.25–2	<0.25	0.5	0.5
	Penicillin G	0.001–0.01	0.001	0.002	0.005
	Cefoxitin	<0.25–2	<0.25	0.5	0.5
Enterococci (18)	LY-127935	32–>500	500	500	500
	Ampicillin	<0.25–4	2	2	2
	Cefoxitin	16–>500	500	500	500
E. coli (29)	LY-127935	<0.25–1	<0.25	0.5	0.5
	Piperacillin	0.5–>500	2	64	>500
	Cephalothin	8–>500	32	64	250
	Cefamandole	0.5–>500	1	64	500
	Cefoxitin	2–16	4	8	8
Klebsiella spp. (36)	LY-127935	<0.25–0.5	<0.25	0.5	0.5
	Piperacillin	4–>500	32	64	250
	Cephalothin	2–64	4	8	16
	Cefamandole	0.5–125	2	4	8
	Cefoxitin	1–16	4	4	8
Enterobacter spp. (32)	LY-127935	<0.25–64	0.5	2	4
	Piperacillin	2–>500	4	4	>500
	Cephalothin	8–>500	>500	>500	>500
	Cefamandole	1–>500	8	500	>500
	Cefoxitin	8–>500	250	500	>500
P. mirabilis (27)	LY-127935	<0.25–4	<0.25	<0.25	4
	Piperacillin	<0.25–1	0.5	0.5	1
	Cephalothin	2–32	4	4	8
	Cefamandole	0.5–8	1	1	2
	Cefoxitin	2–16	4	8	8
Proteus (26)[a]	LY-127935	<0.25–4	<0.25	0.5	0.5
	Piperacillin	<0.25–>500	64	500	500
	Cephalothin	2–>500	>500	>500	>500
	Cefamandole	0.5–>500	64	125	500
	Cefoxitin	2–16	8	8	16
C. freundii (7)	LY-127935	<0.25–4	0.5	2	4
	Piperacillin	32–250	125	125	250
	Cefoxitin	2–16	4	8	16
Serratia spp. (14)[b]	LY-127935	<0.25–8	0.5	1	4
	Piperacillin	0.5–125	1	2	4
	Carbenicillin	4–>500	8	16	16
	Gentamicin	0.5–4	1	2	4
Serratia spp. (20)[c]	LY-127935	0.5–125	2	8	32
	Piperacillin	>500	>500	>500	>500
	Carbenicillin	>500	>500	>500	>500
	Gentamicin	16–>500	64	125	250
P. aeruginosa (30)[b]	LY-127935	16–64	32	32	64
	Piperacillin	4–32	8	8	16
	Carbenicillin	64–250	64	125	125
	Gentamicin	0.5–2	1	1	2
P. aeruginosa (19)[c]	LY-127935	16–32	16	16	32
	Piperacillin	4–>500	32	125	>500
	Carbenicillin	64–500	250	250	500
	Gentamicin	32–>500	500	>500	>500
H. influenzae (14)	LY-127935	<0.001–0.08	0.02	0.04	0.04
	Ampicillin	0.002–250	0.04	0.16	0.16
B. fragilis (26)	LY-127935	<1.0–8	<1.0	<1.0	4
	Cefoxitin	4–16	8	8	8

[a] Indole positive.
[b] Gentamicin susceptible.
[c] Gentamicin resistant.

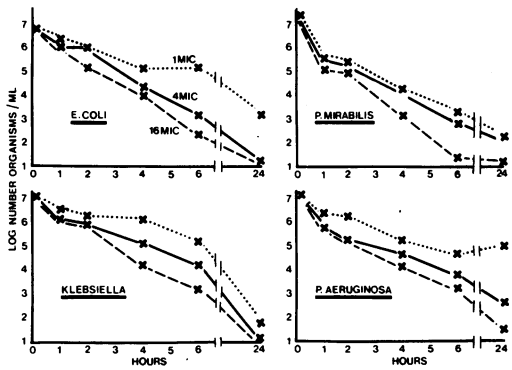

FIG. 1. *Effect of increasing concentrations of LY-127935 on bacterial killing of four gram-negative bacilli.*

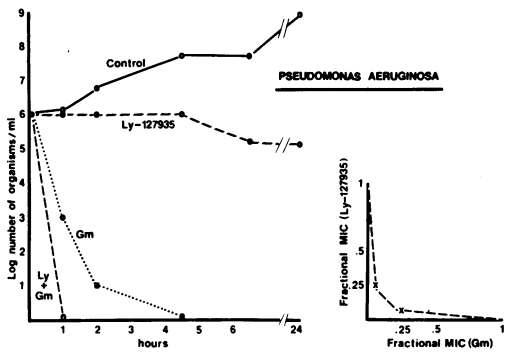

FIG. 2. *Bactericidal effect of LY-127935 (LY) or gentamicin (GM) alone or in combination on Pseudomonas aeruginosa (left). The isobologram is derived from a checkerboard of the combination of LY-127935 plus gentamicin (right), expressed as fractional inhibitory concentrations.*

strains of *P. aeruginosa* was as follows. No decreases (fourfold or greater) in the MIC against any of the strains were observed when 1 or 5 μg of clavulanic acid per ml was added. The MIC of LY-127935 against eight strains of *S. aureus* was decreased by the addition of clavulanic acid at 10 μg/ml (three strains) or 20 μg/ml (five strains). Synergism with the combination was noted with three of the strains of *P. aeruginosa*, but only when 20 μg of clavulanic acid per ml was added.

DISCUSSION

LY-127935, in this investigation, was found to be active against several gram-positive aerobic cocci, but did not have any particular advantage over currently available beta-lactam antibiotics. Its important properties relate to its ability to inhibit the *Enterobacteriaceae*, where it was found to be the most active antibiotic when compared with piperacillin, cephalothin, cefamandole, and cefoxitin. Particularly remarkable was its spectrum against cefamandole-resistant *Enterobacter* spp. and multiple-drug-resistant *Serratia* spp. Inherent in these observations is the apparent stability of LY-127935 against the beta-lactamase activity of various organisms. This is clearly demonstrated with the ampicillin-resistant strain of *H. influenzae*. The anti-pseudomonal properties of LY-127935, especially with gentamicin- and piperacillin-resistant strains, are clearly seen in this study. Synergism was demonstrated for a few strains of *S. aureus* and *P. aeruginosa* with LY-127935 and clavulanic acid. In addition, it is synergistic in combination with gentamicin against enterococci and *P. aeruginosa*, an observation which has been seen with another investigational cephalosporin, HR-756 (2). LY-127935, however, is not synergistic with gentamicin against enterococci at safely achievable serum concentrations. Its increased activity over cefoxitin against *B. fragilis* gives LY-127935 an additional dimension. Thus, LY-127935 has exceptional antibacterial properties which may have clinical significance if pharmacokinetic and toxicological studies demonstrate adequate levels and safety.

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