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LY127935, a Novel Oxa-β-Lactam: an In Vitro Comparison with Other β-Lactam Antibiotics

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The in vitro activities of LY127935 (LY) were compared with those of other β-lactam antibiotics. LY was highly active (minimal inhibitory concentration [MIC] range 0.06 to 0.25 μg/ml) against the common *Enterobacteriaceae* (including *Providencia stuartiia, Enterobacter*, and *Serrati, marcescens*), 8 to 16 times more active than cefoxitin, cefuroxime, or cefazolin, and from one-half to one-eighth as active as cefotaxime (HR756). The activity of LY against *Pseudomonas aeruginosa* (with MICs of 4 and 64 μg/ml for 50 and 90% of test strains, respectively) was essentially similar to that of cefotaxime, but was only one-half as active as CGP 7174/E. LY, cefoxitin, and cefotaxime were essentially equally active against *Bacteroides fragilis*—each was more active than cefuroxime and cefazolin. Against *Staphylococcus aureus*, LY (50% MIC and 90% MIC of 4 and 16 μg/ml, respectively) was less active than cefotaxime, cefoxitin, or cefuroxime and one-eighth as active as cefazolin. The composition and pH of the culture medium had little effect on the activity of LY, although 7.2 appeared to be the optimum pH.

Cephalosporin research has produced compounds of considerable interest such as the β-lactamase-stable cefuroxime and the cephamycin cefoxitin. Cefotaxime (HR756), introduced more recently, has a much broader spectrum of activity and greater potency than other related cephalosporins (3, 8). LY127937 (LY), a novel oxa-β-lactam derivative developed by the Shionogi Co. Osaka, Japan (6059-S), also promises to be of considerable interest (S. Matsuura, T. Yoshida, and S. Kuwahara, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., abstr. no. 152, 1978). This compound, 7-[[carboxy(4-hydroxyphenyl), acetyl]amino]-7-methoxy-3-[[(1-methyl-1H-tetraxol-5-yl)thio]methyl]-8-oxo-5-oxa-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, structurally similar to cefoxitin, has a dihydro-oxazine ring in place of the dihydro-thiazine ring common to cephalosporins and cephamycins. In the current study the activities of LY and other β-lactam antibiotics were compared against a wide range of recent clinical isolates. The novel cephalosporin CGP 7174/E was included in the investigation of the strains of *Pseudomonas aeruginosa* (5).

MATERIALS AND METHODS

Strains and antimicrobial agents. The strains examined in this study are listed in Table 1 according to classification and number. Of the total of 239, 234 were recent clinical isolates from patients in Dudley Road Hospital. Of the remaining five, three were β-lactamase-positive *Neisseria gonorrhoeae* strains obtained from A. Percival (Liverpool, England) and W. A. Ashford (U. S. Air Force). Two strains of *P. aeruginosa* known to be Tem$^+$ were obtained from E. Lowbury (Birmingham, England). All strains were identified by the API (API Laboratory Products Ltd., Farnborough, England) method. The production of β-lactamase by some strains was verified using the chromogenic cephalosporin substrate nitrocefin (4).

The antibiotics were obtained from the following sources: LY and cefazolin from Lilly Research, Windlesham, England; benzylpenicillin, ampicillin, and carbenicillin from Beecham Research Laboratories, Brentford, Middlesex, England, cefoxitin from Merck Sharp & Dohme, Hoddesdon, England; cefuroxime from Glaxo Laboratories, Greenford, Middlesex, England; CGP 7174/E from Ciba Geigy, Horsham, England; and cefotaxime from Roussel Laboratories, Wembley, England.

Methods. The activities of the compounds were studied by a routine agar plate dilution method using Isosensitest agar, pH 7.2 (Oxoid, Basingstoke, England). The Isosensitest agar was supplemented as follows: with 5% lysed human blood to support the growth of *Bacteroides fragilis*, a Levinthal preparation to support the growth of *Haemophilus influenzae*, and a chocolate agar to support the growth of *N. gonorrhoeae*.

Inocula were prepared as follows. For all strains except those of *B. fragilis*, streptococci (including pneumococci), *N. gonorrhoeae*, and *H. influenzae*, the organisms were grown overnight in nutrient broth yielding a viable count of about 10^9 colony-forming units (CFU) per ml. Strains of *B. fragilis* were grown

TABLE 1. *MIC inhibiting cumulative percentage of isolates*[a]

Species	No. of isolates	Antibiotic	MIC range (μg/ml)	50% MIC (μg/ml)	90% MIC (μg/ml)
Escherichia coli	25	LY	0.06–0.25	0.12	0.25
		Cefotaxime	0.015–1.0	0.06	0.12
		Cefoxitin	1–8	2	4
		Cefuroxime	1–8	1	4
		Cefazolin	1–8	1	4
Klebsiella pneumoniae	25	LY	0.06–2	0.12	0.25
		Cefotaxime	0.008–0.25	0.03	0.12
		Cefoxitin	0.5–4	1	2
		Cefuroxime	0.25–16	1	4
		Cefazolin	1–128	1	8
Proteus mirabilis	10	LY	0.06–0.25	0.12	0.25
		Cefotaxime	0.015–0.12	0.015	0.03
		Cefoxitin	1–16	1	2
		Cefuroxime	1–16	1	2
		Cefazolin	1–32	2	4
Indole-positive *Proteus* spp.	10	LY	0.12–0.25	0.12	0.12
		Cefotaxime	0.015–1	0.06	0.12
		Cefoxitin	1–4	1	4
		Cefuroxime	4–>128	16	64
		Cefazolin	64–>128	64	>128
Enterobacter spp.	10	LY	0.06–0.25	0.12	0.12
		Cefotaxime	0.06–0.25	0.06	0.12
		Cefoxitin	64–>128	>128	>128
		Cefuroxime	1–4	2	2
		Cefazolin	1–32	4	8
Salmonella spp. (including 2 *S. typhi*)	5	LY	0.03–0.12	0.06	0.12
		Cefotaxime	0.03–0.06	0.06	0.06
		Cefoxitin	1–2	1	2
		Cefuroxime	1–2	1	2
		Cefazolin	1	1	1
Providencia stuartii	10	LY	0.03–0.06	0.06	0.06
		Cefotaxime	0.06–0.12	0.06	0.12
		Cefoxitin	1–4	1	4
		Cefuroxime	1–4	2	4
		Cefazolin	2–16	8	16
Serratia marcescens	5	LY	0.25	0.25	0.25
		Cefotaxime	0.06–0.25	0.12	0.25
		Cefoxitin	4–8	8	8
		Cefuroxime	8–32	32	32
		Cefazolin	>128	>128	>128
Pseudomonas aeruginosa	35	LY	1–128	4	64
		Cefotazime	4–128	8	64
		Carbenicillin	16–>1028	64	1024
		CGP 7174/E	1–64	2	32
Haemophilus influenzae	25	LY	0.015–0.25	0.06	0.06
		Cefotaxime	0.004–0.03	0.004	0.015
		Ampicillin	0.12–4	0.25	4
		Cefoxitin	1–4	2	4
		Cefuroxime	0.25–1	0.5	1

TABLE 1—*Continued*

Species	No. of isolates	Antibiotic	MIC range (μg/ml)	50% MIC (μg/ml)	90% MIC (μg/ml)
Neisseria gonorrhoeae	25	LY	0.008–1	0.03	0.25
		Penicillin	0.008–1	0.12	1
		Cefoxitin	0.12–0.5	0.25	0.5
		Cefuroxime	0.008–1	0.06	0.5
		Cefazolin	0.12–2	0.5	1
Bacteroides fragilis subsp. *fragilis*	25	LY	0.25–>4	0.5	4
		Cefotaxime	1–64	2	2
		Penicillin	2–512	8	16
		Cefoxitin	2–16	4	8
		Cefuroxime	1–128	4	128
		Cefazolin	2–>128	16	128
Staphylococcus aureus (including 2 methicillin-resistant strains)	24	LY	4–16	4	16
		Cefotaxime	0.5–8	1	8
		Cefoxitin	2–8	2	8
		Cefuroxime	0.5–8	1	4
		Cefazolin	0.12–4	0.25	2

[a] Inoculum, 10^3 CFU.

overnight in thioglycolate broth, streptococci were grown in Todd-Hewitt broth, and *H. influenzae* strains were grown in Levinthal broth yielding about the same viable counts. Strains of *N. gonorrhoeae* were grown overnight on a chocolate agar plate, the growth being removed just before use and resuspended in peptone water so as to yield a viable count of 10^6 to 10^7 CFU/ml. Two inocula, 10^3 and 10^6 CFU/ml, were employed in the sensitivity tests of all organisms except *N. gonorrhoeae*. These were obtained by transferring 1 μl undiluted and a 1:1,000 dilution of the overnight culture to the surface of the antibiotic-containing agar by a Denley (Denley-Tech Ltd., Billingshurst, England) multipoint inoculating device. Strains of *N. gonorrhoeae* were tested undiluted and at a 1:10 dilution, thus yielding final inocula of 10^3 to 10^4 and 10^2 to 10^3 CFU.

All plates were incubated in air at 37°C, with the exception of the anaerobes which were incubated in a GasPak jar (BBL Microbiology Systems, Cockeysville, Md.) and *H. influenzae* and *N. gonorrhoeae* which were incubated in a 10% CO_2 incubator. The period of incubation was 24 h. The minimal inhibitory concentration (MIC) of the antibiotic was defined as the micrograms per milliliter of medium at which there was an estimated 99% reduction (by counting) in the original inoculum.

The influence of different media upon the MIC of LY was evaluated using five strains each of *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Providencia stuartii*, *Staphylococcus aureus*, indole-positive and -negative *Proteus* spp., *P. aeruginosa*, *Serratia marcescens*, and Lancefield group D streptococci. The media chosen were as follows: Isosensitest, pH 7.2, 6.0, and 8.0; Wellcotest, pH 7.2 (Wellcome Research Laboratory, Beckenham, England); SAF, pH 7.2 (Mast Laboratory, Liverpool, England); Penassay no. 1., pH 6.6 (Oxoid Ltd.), Penassay no. 5, pH 8.0; and Meuller-Hinton, pH 7.3 (Difco Laboratories, Detroit, Mich.). The two inocula and incubation conditions described above were employed.

RESULTS

Table 1 summarizes the results obtained from the 234 isolates tested at an inoculum of 10^3 CFU. LY had a high degree of activity against the *Enterobacteriaceae*, including known β-lactamase-producing strains, showing about one-half the activity of cefotaxime but being at least 8- to 16-fold more active than cefoxitin, cefuroxime, and cefazolin. The clinically important genera tested, *Providencia* and *Serratia*, also were highly susceptible to LY and cefotaxime. It was noted that, when a strain showed decreased susceptibility to cefotaxime, this was reflected in decreased susceptibility to LY and cefoxitin, for example, an *E. coli* with an MIC to cefotaxime of 1 μg/ml, to LY of 0.25 μg/ml, and to cefoxitin of 8 μg/ml.

Among the *Enterobacteriaceae*, an increase in inoculum to 10^6 CFU resulted in at most a two-fold decrease in susceptibility to LY, cefotaxime, cefoxitin, and cefuroxime.

LY and cefotaxime were essentially equally active against *P. aeruginosa* but only one-half as active as CGP 7174/E. Tem^+ and Tem^- strains were equally susceptible to LY and cefotaxime.

LY was clearly more active than ampicillin, cefoxitin, and cefuroxime against *H. influenzae* but was only one-fourth to one-sixteenth as active as cefotaxime. The eight β-lactamase-producing strains were as susceptible to LY and cefotaxime as were the non-β-lactamase producers. Whereas an increase in inoculum of β-lactamase producers had little effect on the activity of LY, it decreased the activity of ampicillin markedly.

The activity of LY against *N. gonorrhoeae* was similar to that of cefuroxime, non-β-lactamase producers being equally susceptible to LY and penicillin. The β-lactamase producers were penicillin resistant (MIC > 1 μg/ml), and two of these three strains were susceptible to LY and cefuroxime (MIC < 1 μg/ml). One strain (from Liverpool) had an MIC of LY and cefuroxime of 1 μg/ml.

The *B. fragilis* strains were four- to eight-fold more susceptible to LY than to cefoxitin or cefotaxime at the 50% MIC endpoint. Four of the 25 strains had an MIC to LY of ≥4 μg/ml. An eightfold decrease in susceptibility to cefotaxime was noted on increasing the inoculum to 10^6 CFU, but only a twofold decrease was encountered with LY and cefoxitin.

LY was less active against *S. aureus* than the three other cephalosporins, whereas Lancefield group A streptococci (two strains tested, not shown in the table) and *Streptococcus pneumoniae* (three strains tested) were susceptible (LY MIC of 0.25 to 0.5 μg/ml). Lancefield group D streptococci (five strains tested) were highly resistant, having an MIC > 16 μg/ml.

A study of the effects of culture medium upon the MIC of LY, involving work with 45 isolates, revealed only nil or one discrepant result (defined as an MIC of greater than twofold compared with the reference medium, Isosensitest, pH 7.2) with the following media: Wellcotest, SAF, Meuller-Hinton, and Isosensitest (pH 8.0). Penassay no. 5 (pH 8) and Isosensitest (pH 6.0) showed 4 and 5 discrepant results, respectively, and Penassay no. 1 (pH 6.6) showed 10 discrepant results. In all cases the MICs of LY obtained in the discrepant results were higher than those on the reference media. It appeared that the optimum pH for the in vitro activity of LY was 7.2.

DISCUSSION

LY, a structurally novel β-lactam, shares many properties with the cephalosporins. This study shows that, with the exception of *S. aureus*, LY is more active than cefoxitin or cefuroxime and hence more active than cefamandole (2). LY shares with cefotaxime a high degree of activity against the *Enterobacteriaceae* (8) including the "problem" organisms of hospital practice such as *Serratia*, *P. stuartii*, and the *Enterobacter* spp. The activity of LY against *P. aeruginosa* is similar to that of cefotaxime but less than the antipseudomonal cephalosporin CGP 7174/E. It would appear that LY is stable to β-lactamase hydrolysis as little decrease in susceptibility was noted on increasing the inoculum.

Against *B. fragilis*, LY was four to eight times more active than cefoxitin, an agent of proven clinical usefulness in anaerobic sepsis (6). Four strains of decreased susceptibility (two of which were highly penicillin resistant) were encountered, but little inoculum effect was noted. Whether this resistance is related to poor cell penetration or β-lactam hydrolysis is not known. In the case of cefotaxime, both mechanisms are probably involved (1, 7).

Against gram-positive pathogens, LY had activity similar to that of cefotaxime, namely, *S. aureus* strains were relatively less susceptible, fecal streptococci were highly resistant, and group A streptococci and pneumococci were susceptible. *N. gonorrhoeae* and *H. influenzae*, including the clinically important β-lactamase-producing strains, were highly susceptible.

Studies in experimental animals suggest that LY will need to be administered parenterally. The compound is excreted in a form microbiologically indistinguishable from the parent compound, unlike cefotaxime which undergoes considerable metabolism to the desacetyl form (P. Johnson, R. Glomot, and M. Kramer, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., abstr. no. 81, 1978). Preliminary data (R. Wild, personal communication) suggest that high serum levels are achieved and the serum half-life is relatively long. Thus, administration of relatively small doses of LY might be efficacious in the treatment of the common gram-negative infections; higher doses would probably be needed for treatment of *S. aureus* or *P. aeruginosa* infections.

LY has a novel structure, an extremely interesting antimicrobial spectrum, and a wide potential demanding clinical study.

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