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In Vitro Activities of LY163892, Cefaclor, and Cefuroxime

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The in vitro activity of LY163892, a synthetic oral cephalosporin, was compared with those of cefaclor and cefuroxime against 1,193 clinical isolates. MIC ranges and MICs for 50 and 90% of isolates of the three cephalosporins were comparable. The activities of LY163892 and cefaclor were, however, highly inoculum dependent against β-lactamase-positive *Haemophilus influenzae* and *Staphylococcus aureus*; that of cefuroxime was not. LY163892 and cefuroxime appeared stable in microdilution trays stored at 5 and −20°C for 5 weeks, in contrast to cefaclor which remained stable for more than a week only at −5°C.

Cefaclor was previously shown to be active against staphylococci, pneumococci, nonenterococcal streptococci, neisseriae, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, salmonellae, shigellae, *Citrobacter diversus*, and, at inocula in the high 10^4-CFU/ml to low 10^5-CFU/ml range, β-lactamase-positive and -negative *Haemophilus influenzae* (1, 7); however, the compound has been shown to lose activity rapidly in pH 7.0 and 8.0 buffers, as well as in plasma and serum, at 5, 25, and 37°C (3). LY163892 is a novel, synthetic, orally administered cephalosporin {7-[D-(aminophenylacetyl)amino]-3-chloro-8-oxo-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid} that is similar in structure and activity to cefaclor but that appears to have greater stability in vitro than cefaclor.

The purposes of this study were (i) to compare the antibacterial activities of LY163892, cefaclor, and cefuroxime, which has increased stability to plasmid-mediated β-lactamases (8, 9) and from which has been developed an orally administered ester, cefuroxime axetil (4); (ii) to determine inoculum effects of β-lactamase-producing *H. influenzae* and *Staphylococcus aureus* on LY163892, cefaclor, and cefuroxime; and (iii) to assess the stabilities of LY 163892, cefaclor, and cefuroxime under refrigerated and frozen storage conditions.

A total of 1,193 clinical isolates were tested by a broth microdilution method, as recommended by the National Committee for Clinical Laboratory Standards (6). With the exception of a number isolates of *H. influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae*, which were kindly supplied by Michael Jacobs, University Hospitals of Cleveland, and Clyde Thornsberry of the Centers for Disease Control, all strains tested were clinical isolates from the Bacteriology Section in the Department of Microbiology at the Cleveland Clinic Foundation. Control strains included *S. aureus* ATCC 29213, *E. coli* ATCC 25922, and *Enterococcus* (*Streptococcus*) *faecalis* ATCC 29212. All MICs, except for *Haemophilus* sp. and *S. pneumoniae*, were determined in cation-supplemented Mueller-Hinton broth with an inoculum of approximately 5×10^5 CFU/ml. MICs for *Haemophilus* sp. and *S. pneumoniae* were determined with an inoculum of approximately 3×10^5 CFU/ml in Mueller-Hinton broth supplemented with 15 μg of bovine hematin per ml, 15 μg of NAD per ml, and 5 mg of yeast extract per ml (J. H. Jorgensen, J. S. Redding, L. A. Maher, and A. W. Howell, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, C-219, p. 359).

Inoculum effects of 10 β-lactamase-positive strains of *H. influenzae* and 10 β-lactamase-positive strains of *S. aureus* were tested by determining MICs of each antibiotic against each strain at inocula of approximately 3×10^5 and 3×10^7 CFU/ml.

For stability studies, 20 microdilution trays containing LY163892, cefaclor, and cefuroxime in serial $\log_2$ dilutions were prepared, and 10 trays each were stored at 5 and −20°C. Two trays from each storage condition were removed each week, allowed to adjust to room temperature, and inoculated with *S. aureus* ATCC 29213 and *E. coli* ATCC 25922.

MICs for groups, genera, or species represented by nine or more strains each are listed in Table 1, and MICs for groups, genera, or species represented by five or fewer strains each are listed separately in Table 2. LY163892, cefaclor, and cefuroxime MIC ranges and MICs for 50 and 90% of isolates were the same for β-lactamase-positive and β-lactamase-negative strains of *H. influenzae* and are, therefore, not listed separately in Table 1. MICs of LY163892, cefaclor, and cefuroxime against a β-lactamase-negative, ampicillin-resistant strain of *H. influenzae* were 64, >64, and 16 μg/ml, respectively. Of a total of 23 methicillin-resistant staphylococci tested, 4 were inhibited by 16 μg and 1 was inhibited by 32 μg of LY163892 per ml, while the remainder were resistant to 64 μg of LY163892 per ml. MICs of cefaclor and cefuroxime were within control limits for *S. aureus* ATCC 29213, *E. coli* ATCC 25922, and *E. faecalis* ATCC 29212; the corresponding MICs of LY163892 were, respectively, 1 to 2, 0.5 to 1, and 64 μg/ml.

LY163892 and cefaclor had inoculum-dependent activity against β-lactamase-positive *H. influenzae* and *S. aureus* (Table 3). No inoculum effect was seen with cefuroxime when it was tested against these same strains. These observations may reflect the greater stability of cefuroxime relative to those of cefaclor and, presumably, LY163892 to staphylococcal and *H. influenzae* β-lactamases (2, 5, 8, 9).

In the stability studies, MICs of LY163892 and cefuroxime remained within control limits against *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 during the 5 weeks of testing trays stored at 5 and −20°C. MICs of cefaclor exceeded control limits for *S. aureus* ATCC 29213 when trays stored at 5°C for 2 or more weeks were tested and for *E. coli* ATCC 25922 when trays stored at 5°C for 3 or more weeks were

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TABLE 1. Comparison of in vitro activities of LY163892, cefaclor, and cefuroxime

Organism (no. of isolates)	MIC (μg/ml)[a]								
	LY163892			Cefaclor			Cefuroxime		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Staphylococcus aureus (112)	1–>64	4	>64	0.5–>64	2	>64	1–>64	2	>64
Staphylococcus spp., coagulase negative (109)	0.25–>64	4	>64	0.25–>64	2	64	0.12–>64	1	>64
Streptococcus spp., group A (22)	≤0.06–2	≤0.06	≤0.06	≤0.06–0.5	≤0.06	≤0.06	≤0.06–0.12	≤0.06	≤0.06
Streptococcus spp., group B (24)	≤0.06–2	1	1	≤0.06–0.5	0.25	0.5	≤0.06–0.12	0.06	≤0.06
Streptococcus spp., group D (89)	4–>64	64	>64	0.5–>64	64	>64	≤0.06–>64	>64	>64
Streptococcus spp., other groups (14)	≤0.06–4	0.12	4	≤0.06–2	≤0.06	1	≤0.06–2	≤0.06	0.12
Streptococcus pneumoniae (17)	≤0.06–4	0.25	1	≤0.06–1	0.12	0.25	≤0.06–0.25	≤0.06	≤0.06
Branhamella catarrhalis (17)	≤0.12–2	1	1	≤0.06–1	0.5	1	0.12–2	0.5	1
Neisseria meningitidis (11)	≤0.06–0.12	≤0.06	0.12	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06
Haemophilus influenzae (29)	0.12–64	1	2	≤0.06–>64	1	2	≤0.06–16	0.25	2
Escherichia coli (229)	≤0.06–>64	1	4	0.25–>64	1	4	0.5–>64	1	4
Klebsiella oxytoca (26)	0.5–>64	1	>64	≤0.06–>64	0.5	>64	0.12–>64	1	>64
Klebsiella pneumoniae (93)	0.12–>64	0.5	1	≤0.06–>64	0.5	1	0.25–>64	1	8
Enterobacter cloacae (56)	0.5–>64	64	>64	0.5–>64	>64	>64	0.5–>64	8	>64
Enterobacter aerogenes (29)	32–>64	>64	>64	64–>64	>64	>64	0.5–>64	>64	>64
Serratia marcescens (26)	64–>64	>64	>64	64–>64	>64	>64	8–>64	32	>64
Citrobacter diversus (9)	0.25–0.5	0.5	0.5	0.25–0.5	0.25	0.5	2–32	2	32
Citrobacter freundii (38)	0.5–>64	64	>64	0.5–>64	>64	>64	1–>64	64	>64
Proteus mirabilis (53)	1–>64	1	2	0.25–>64	0.5	1	0.25–>64	0.5	1
Morganella morganii (16)	64–>64	>64	>64	>64	>64	>64	2–>64	32	64
Pseudomonas aeruginosa (109)	>64	>64	>64	>64	>64	>64	>64	>64	>64
Pseudomonas maltophilia (11)	>64	>64	>64	>64	>64	>64	>64	>64	>64
Acinetobacter anitratus (15)	32–>64	>64	>64	32–>64	>64	>64	16–>64	32	64

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.

TABLE 2. MICs of LY163892, cefaclor, and cefuroxime against species represented by five or fewer clinical isolates each

Organism	MICs (μg/ml) for individual isolates[a]		
	LY163892	Cefaclor	Cefuroxime
Acinetobacter lwoffii	0.5, 1, 4, 32, 64	0.5, 1, 4, 32, 64	2, 4, 8, 16, 32
Aeromonas hydrophila	64_4, >64	$>64_5$	1_2, 2_2, 8
Alcaligenes sp.	0.5_2, 16	0.25, 0.5, 32	64_2, >64
CDC group 17	64_2	$>64_2$	1, 2
CDC group Ve-2	64_4, >64	64, $>64_4$	16_2, 32_2, 64
CDC group Ve-1	64	>64	32
Haemophilus parainfluenzae	≤0.06, 0.5	≤0.06, 0.5	≤0.06, 0.25
Citrobacter amalonaticus	0.5	0.25	2
Enterobacter agglomerans	0.5	0.5	1
Enterobacter sakazakii	0.5, 64	2, >64	0.5, 2
Hafnia alvei	64	>64	64
Proteus vulgaris	64_4	$>64_4$	$>64_4$
Providencia stuartii	2_2, 32, >64	4_2, 64, >64	0.5_2, 1, 32
Serratia liquefaciens	>64	>64	>64
Pseudomonas sp.	0.5_2, 64_2, >64	2_2, $>64_3$	1_2, 64_2, >64

[a] The inferior number indicates the number of isolates for which the MIC was as indicated.

TABLE 3. Inoculum-related activities of LY163892, cefaclor, and cefuroxime against 10 strains each of β-lactamase-positive *H. influenzae* and *S. aureus*

Organism	Inoculum (CFU/ml)	MIC (μg/ml)[a]								
		LY163892			Cefaclor			Cefuroxime		
		Range	50%	90%	Range	50%	90%	Range	50%	90%
H. influenzae	3×10^5	0.12–1	1	1	≤0.06–1	0.5	1	≤0.06–0.25	0.25	0.25
	3×10^7	2–16	4	4	1–16	4	4	≤0.06–2	0.25	0.25
S. aureus	5×10^5	4–16	4	16	1–32	2	32	1–2	2	2
	5×10^7	8–>64	16	>64	4–>64	>64	>64	1–2	1	2

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.

tested. MICs of cefaclor remained within control limits for both control strains over the course of 5 weeks with trays stored at −20°C.

In conclusion, the spectrum of activity of LY163892 is comparable to those of cefaclor and, in many respects, cefuroxime. In contrast to that of cefuroxime, the activities of both LY163892 and cefaclor were adversely affected by increased inocula of β-lactamase-positive *H. influenzae* and *S. aureus*. The stability of LY163892 was considerably greater than that of cefaclor under 5°C storage conditions.

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