In Vitro Antibacterial Activity of a
New 1-Oxa Cephalosporin Compound

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The *in vitro* activity of a unique new 1-oxa cephalosporin beta-lactam antibiotic (LY 127935) was tested against clinical isolates of gram-positive and gram-negative bacteria and compared with the activities of cefoxitin, cefamandole, cephalothin, clindamycin, amikacin, tobramycin, gentamicin, ticarcillin, and carbenicillin. The new compound was observed to have a broad spectrum of antibacterial activity which far exceeded the activity of older cephalosporins against aerobic gram-negative enteric bacilli. This new compound was the most active drug tested against *Klebsiella*, *Serratia*, *Enterobacter*, indole-negative and positive *Proteus* species, and *E. coli*. Against clinical isolates of Pseudomonas species the new compound was more active than cefoxitin, cefamandole, cephalothin, and clindamycin, comparable to ticarcillin and carbenicillin, and less active than gentamicin, tobramycin, and amikacin. Yet, most of the *Pseudomonas* isolates were inhibited by 16 ug/ml of the new compound. Against both beta-lactamase and non beta-lactamase producing *Staphylococcus aureus* isolates, the new 1-oxa compound was less active than the older cephalosporins of which cephalothin and cefamandole were the most effective. The 1-oxa compound had no appreciable activity against isolates of *Streptococcus faecalis*. Activity of all four cephalosporins studied was decreased in the presence of an increased inoculum of *Enterobacteriaceae* in trypticase soy and Mueller-Hinton broth. The activity of the new compound against *Pseudomonas species* was also decreased by an increased inoculum in Mueller-Hinton but not in trypticase soy broth. These results indicate that this new 1-oxa compound may have great promise as a broad spectrum antibiotic and may warrant controlled clinical trials in man.

Recently, a number of new cephalosporins have been introduced which have expanded the antibacterial spectrum of this class of antimicrobial agents [1,2]. One of these compounds, (6R, 7R)-7-[(Carboxy(4-hydroxyphenyl) acetyl) amino]-7-methoxy-3-[(1-methyl-1H-tetrazol-5-y1) thio) methyl]-8-oxo-5 oxa-1 azabicyclo-[4.2.0] oct-2-ene-2 carboxylic acid (LY 127935), is a new beta lactam antibiotic. Structurally, this compound contains a conventional cephem nucleus in which oxygen is substituted in place of sulfur in the six-membered ring [3].

The purpose of this study was to characterize more thoroughly the *in vitro* activity of this new antibiotic against a wide variety of gram-negative and gram-positive organisms isolated from patients and to compare their activity to the antimicrobial activity of agents currently in clinical use. Specifically, the *in vitro* activity of LY 127935 was compared to that of cefoxitin, cephalothin, cefamandole, carbenicillin, ticarcillin, gentamicin, tobramycin, amikacin, and clindamycin.

MATERIALS AND METHODS

Antibiotics

Ten antibacterial agents were tested and were kindly supplied as laboratory standards by their manufacturers. Gentamicin was supplied by Schering Corporation, Bloomfield, NJ; tobramycin, cefamandole, keflin, LY 127935 were forwarded from Eli Lilly and Company, Indianapolis, IN; amikacin came from Bristol Laboratories, Syracuse, NY; cefoxitin was supplied by Merck, Sharpe and Dohme, West Point, PA; ticarcillin was supplied by Beecham Laboratories, Bristol, TN; carbenicillin came from Roerig, New York, NY; and clindamycin was supplied by Upjohn Company, Kalamazoo, MI. Dilutions of the antibiotic were prepared fresh in sterile growth medium and used the same day.

Bacterial Strains

Fifteen strains each of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Serratia marscescens*, *Enterobacter species*, indole positive *Proteus*, indole negative *Proteus*, penicillin sensitive *Staphylococcus aureus*, penicillin resistant *Staphylococcus aureus*, and Group D *Streptococci* were clinical isolates used in this study. These strains were obtained from patients hospitalized within the past year at Yale–New Haven Hospital and the West Haven Veterans Administration Hospital. All fifteen strains of penicillin resistant *S. aureus* showed minimal inhibitory concentrations (MIC) of penicillin (Eli Lilly and Co., Indianapolis, IN) equal to or greater than 512 ug/ml as determined with the microtiter method outlined below. Penicillin sensitivity among *S. aureus* strains was defined by a zone of inhibition of at least 29 mm around a 10 IU penicillin impregnated disc, using standard *Kirby-Bauer* technique [4].

In Vitro Testing

For MICs, eleven twofold serial dilutions of each antimicrobial in trypticase soy broth (TSB) (BBL, Inc., Cockeysville, MD) were prepared [5,6]. The highest concentrations of each drug tested were as follows: gentamicin and tobramycin, 32 ug/ml; amikacin, cefoxitin, cephalothin, cefamandole, and LY 127935, 128 ug/ml; ticarcillin and carbenicillin, 512 ug/ml; clindamycin, 64 ug/ml. The dilutions of each antimicrobial were applied to a $3\frac{3}{8} \times 4\frac{1}{4}$ inch microtiter plate containing 96 wells arranged in eight rows of twelve wells each. Each well was loaded with 0.1 ml of the appropriate drug dilution with a semi-automated pipettor (Dynatech, Inc., Alexandria, VA). One column of wells on each plate contained drug-free medium as a growth control. Each well was inoculated with 10^4 colony forming units (10^5 cfu/ml) using an automated inoculator (Dynatech, Inc., Alexandria, VA). Eighteen-hour broth cultures of each organism were adjusted by visible light colorimetry in order that proper final inoculum densities were obtained. At the end of 18–24 hours' incubation at 37°C, duplicate rows for each strain were compared and MICs recorded as the lowest concentration of antibiotic which showed no visual turbidity. Where duplicate rows differed by only one well, MICs were reported as the higher concentration. Duplicates that differed by more than one well were repeated until consistent results were obtained. Minimal bactericidal concentrations (MBC) were determined by transferring an aliquot from each well of an MIC plate to trypticase soy agar (TSA) (BBL, Inc., Cockeysville, MD) poured into single-well $3\frac{3}{8} \times 4\frac{1}{4}$ inch trays with the automated inoculating system previously described. The MBC was the

concentration at which no colonial growth appeared after 24 hours of incubation at 37°C.

Inoculum Density and Media Testing

In order to determine effects of varying inoculum cell density and media, cephalothin, cefoxitin, cefamandole, and LY 127935 vs. all 150 organisms were tested in the following combinations: *(1)* 1×10^7 cfu/ml in TSB; *(2)* 1×10^5 cfu/ml in Mueller-Hinton broth (MHB); *(3)* 1×10^7 cfu/ml in MHB as well as with 1×10^5 cfu/ml in TSB as described above and MICs and MBCs were determined.

RESULTS

The comparative *in vitro* activities of the 1-oxa cephalosporin and other antimicrobical agents are shown in Table 1. Against *Pseudomonas* species the 1-oxa compound was more active than cefoxitin, cefamandole, cephalothin, and clindamycin, was comparable in activity to ticarcillin and carbenicillin, but was less active than the aminoglycosides, gentamicin, tobramycin, and amikacin. The 1-oxa compound was also more active than cefoxitin, cefamandole, cephalothin, clindamycin, amikacin, tobramycin, gentamicin, ticarcillin, and carbenicillin against *Klebsiella, Serratia, Enterobacter*, and both indole negative and positive *Proteus* species, as well as *E. coli.* Furthermore, our results indicate that the 1-oxa compound inhibited members of the *Enterobacteriaceae* at lower concentrations than did the older cephalosporin, cephalothin, or the newer ones, cefamandole and cefoxitin. In fact, the new 1-oxa cephalosporin compound was the most active agent tested against *Klebsiella, Serratia, Enterobacter*, indole negative and positive *Proteus* species, and *E. coli.* For example, the new compound inhibited 50 and 90 percent of: *Klebsiella* species isolates at concentrations of 0.25 and 0.5 ug/ml, respectively; *Serratia* species isolates at concentrations of 0.5 and 8 ug/ml respectively; *Enterobacter* species isolates at concentrations of 0.25 and 4.0 ug/ml, respectively; indole negative *Proteus* species isolates at concentrations of *less than* 0.12 ug/ml; indole positive *Proteus* species isolates at concentrations of *less than* 0.12 and 0.25 ug/ml, respectively; and *E. coli* isolates at concentrations of *less than* 0.12 and 0.5 ug/ml, respectively. No other antibiotic tested in this study demonstrated such a high degree of *in vitro* activity against these bacterial isolates. However, the new compound was less active than the aminoglycosides against *Pseudomonas* species isolates. For example, 50 percent of the Pseudomonas isolates tested were inhibited by 16 ug/ml of the new compound, a concentration comparable to carbenicillin, ticarcillin and gentamicin, but more than the concentration of tobramycin (2.0 ug/ml) or amikacin (4.0 ug/ml) required to inhibit the same number of isolates. Similarly, 90 percent of the *Pseudomonas* isolates were inhibited by the 1-oxa compound at a concentration of 128 ug/ml or less, which was lower than the concentrations of all other antimicrobial agents studied except for the aminoglycosides, and of these, tobramycin, at a concentration of 2.0 ug/ml, was the most active.

Against non-beta lactamase producing isolates of *Staphylococcus aureus* the new compound was less active than cefamandole, cephalothin, clindamycin, tobramycin, and carbenicillin, and was comparable in activity to cefoxitin, amikacin, gentamicin, and ticarcillin. In fact, cephalothin and cefamandole were the most active agents against these staphylococcal isolates as well as against the beta-lactamase producing isolates of *Staphylococcus aureus* which were tested in this study. The new compound was more active against these beta-lactamase producing staphylococcal

TABLE 1

Comparative In Vitro Activity of 1-Oxa Cephalosporin and Other Antibiotics in
Trypticase Soy Broth with an Inoculum of 1×10^5 cfu/ml

Organism (No. of strains)	Drug	Range of MIC (ug/ml)	50% MIC (ug/ml)	90% MIC (ug/ml)
Pseudomonas sp. (15)	Carbenicillin	16.0–>512	32	>512
	Ticarcillin	8.0–512	16	512
	Gentamicin	1.0–8.0	8.0	8.0
	Tobramycin	0.5–4.0	2.0	2.0
	Amikacin	2.0–8.0	4.0	8.0
	Clindamycin	>64.0	>64	>64
	Cephalothin	>128	>128	>128
	Cefamandole	>128	>128	>128
	Cefoxitin	128–>128	>128	>128
	1-Oxa	16.0–128	16	128
Klebsiella sp. (15)	Carbenicillin	64.0–>512	256	>512
	Ticarcillin	64.0–>512	256	>512
	Gentamicin	1.0–4.0	2.0	4.0
	Tobramycin	2.0–>32	4.0	4.0
	Amikacin	2.0–8.0	4.0	8.0
	Clindamycin	>64.0	>64	>64
	Cephalothin	2.0–8.0	2.0	8.0
	Cefamandole	0.5–4.0	1.0	2.0
	Cefoxitin	4.0–16.0	4.0	8.0
	1-Oxa	<0.1–0.5	0.25	0.5
Serratia sp. (15)	Carbenicillin	2.0–>512	4.0	>512
	Ticarcillin	4.0–>512	4.0	>512
	Gentamicin	1.0–>32	4.0	8.0
	Tobramycin	4.0–>32	8.0	>32
	Amikacin	2.0–16.0	8.0	16
	Clindamycin	>64.0	>64	>64
	Cephalothin	16.0–>128	>128	>128
	Cefamandole	4.0–>128	32	64
	Cefoxitin	16.0–>128	32	128
	1-Oxa	0.2–32.0	0.5	8.0
Enterobacter sp. (15)	Carbenicillin	2.0–128	4.0	32
	Ticarcillin	<0.5–128	4.0	64
	Gentamicin	1.0–16.0	2.0	4.0
	Tobramycin	2.0–8.0	4.0	8.0
	Amikacin	2.0–32.0	4.0	16
	Clindamycin	>64.0	>64	>64
	Cephalothin	8.0–>128	>128	>128
	Cefamandole	1.0–>128	4.0	>128
	Cefoxitin	32.0–>128	>128	>128
	1-Oxa	<0.1–16.0	0.25	4.0
Proteus sp. (15) (Indole neg.)	Carbenicillin	<0.5–5.2	<0.5	8.0
	Ticarcillin	<0.5–64	1.0	32
	Gentamicin	2.0–8.0	4.0	8.0
	Tobramycin	1.0–8.0	4.0	8.0
	Amikacin	4.0–16.0	4.0	16
	Clindamycin	>64.0	>64	>64
	Cephalothin	2.0–128	8.0	32
	Cefamandole	0.5–>128	2.0	4.0
	Cefoxitin	4.0–>128	8.0	16
	1-Oxa	<0.1–0.2	<0.12	<0.12

TABLE 1
Continued

Organism (No. of strains)	Drug	Range of MIC (ug/ml)	50% MIC (ug/ml)	90% MIC (ug/ml)
Proteus sp. (15)	Carbenicillin	≤0.5–16.0	1.0	8.0
(Indole pos.)	Ticarcillin	<0.5–32.0	<0.5	16
	Gentamicin	2.0–32.0	8.0	32
	Tobramycin	1.0–8.0	4.0	8.0
	Amikacin	2.0–128	4.0	16
	Clindamycin	32.0->64.0	>64	>64
	Cephalothin	>128	>128	>128
	Cefamandole	1.0->128	2.0	>128
	Cefoxitin	4.0->128	16	32
	1-Oxa	<0.1–0.2	<0.12	0.25
S. aureus (15)	Carbenicillin	≤0.5–2.0	<0.5	2.0
(Penicillin sensitive)	Ticarcillin	1.0–4.0	1.0	4.0
	Gentamicin	2.0–8.0	4.0	8.0
	Tobramycin	1.0–8.0	1.0	2.0
	Amikacin	8.0–16.0	16	16
	Clindamycin	<0.06–0.12	0.12	0.12
	Cephalothin	<0.12–0.25	0.25	0.25
	Cefamandole	<0.12–0.25	0.25	0.25
	Cefoxitin	4.0	4.0	4.0
	1-Oxa	<0.1–8.0	8.0	8.0
S. aureus (15)	Carbenicillin	<0.5–4.0	2.0	4.0
(Penicillin resistant)	Ticarcillin	2.0–8.0	4.0	8.0
	Gentamicin	0.25->32	1.0	>32
	Tobramycin	0.5->32	1.0	>32
	Amikacin	2.0–128	8.0	128
	Clindamycin	<0.06->64	<0.06	>64
	Cephalothin	<0.12–0.25	0.25	0.25
	Cefamandole	0.25–4.0	0.25	0.5
	Cefoxitin	1.0–2.0	1.0	2.0
	1-Oxa	4.0–8.0	4.0	8.0
E. coli (15)	Carbenicillin	2.0–8.0	4.0	8.0
	Ticarcillin	2.0->512	4.0	8.0
	Gentamicin	2.0–8.0	4.0	4.0
	Tobramycin	4.0–8.0	4.0	8.0
	Amikacin	2.0–8.0	8.0	8.0
	Clindamycin	>64.0	>64	>64
	Cephalothin	8.0–32	8.0	16
	Cefamandole	0.25–4.0	1.0	2.0
	Cefoxitin	8.0–128	8.0	32
	1-Oxa	<0.1–1.0	<0.12	0.5
Enterococci (15)	Carbenicillin	16.0–32	16	16
	Ticarcillin	32.0–64	32	64
	Gentamicin	32.0->32	>32	>32
	Tobramycin	32.0->32	>32	>32
	Amikacin	64.0->128	>32	>32
	Clindamycin	8.0->64	32	>64
	Cephalothin	8.0–32	16	16
	Cefamandole	8.0–32	16	32
	Cefoxitin	>128	16	32
	1-Oxa	>128	>128	>128

isolates than were the aminoglycosides and clindamycin, but was less active than the other cephalosporins including cefoxitin. Furthermore, the 1-oxa compound had poor inhibitory activity against *Streptococcus faecalis* and was considerably less active than the older cephalosporin antibiotics. Specifically, concentrations of 128 ug/ml of the new compound failed to inhibit the growth of any of the *S. faecalis* isolates tested in this study.

Effect of inoculum size and growth medium. The effect of inoculum size on the minimum inhibitory concentration of the cephalosporin compounds tested in this study is shown in Table 2. In these experiments the inoculum size was increased a hundredfold to 10^7 cfu per ml in trypticase soy broth (TSB). Higher concentrations of each of the four cephalosporins were required to achieve inhibition of growth of the larger inoculum of the *Enterobacteriaceae* isolates tested in this study although no differences in MICs were observed against *Pseudomonas species* isolates. Similarly, the *in vitro* activity of these four cephalosporin antibiotics was not significantly increased by a hundredfold increase in inoculum size of both beta-lactamase and non beta-lactamase producing strains of *Staphylococcus aureus*. The higher inoculum of *Streptococcus faecalis* isolates resulted in the loss of inhibitory activity of cefoxitin and only slightly influenced the activity of cefamandole and cephalothin against these bacterial strains. The new 1-oxa compound had no detectable activity against the isolates of *Streptococcus faecalis* either at the lower or higher inoculum tested in this study.

A comparison of the minimum inhibitory concentrations of these four cephalosporins in Mueller-Hinton broth at both the lower (10^5 cfu per ml) and higher (10^7 cfu per ml) inocula is shown in Tables 3 and 4. Again the activity of the new 1-oxa compound, as well as that of cefoxitin, cefamandole, and cephalothin was decreased against *Klebsiella*, *Serratia*, *Enterobacter*, indole negative and indole positive *Proteus* species, and *E. coli* in the presence of the higher inoculum of 1×10^7 cfu per ml in Mueller-Hinton broth as it was in trypticase soy broth (Tables 1 and 2). However, in contrast to the results observed in trypticase soy broth the activity of the 1-oxa compound against *Pseudomonas* species isolates was decreased by the increased inoculum in Mueller-Hinton broth (Tables 2 and 4). Furthermore, the activity of all four cephalosporin antibiotics was also decreased by the increased inoculum of non beta-lactamase producing *Staphylococcus aureus* isolates in Mueller-Hinton broth but was not influenced by the larger inoculum of beta-lactamase producing strains (Tables 3 and 4). This effect was not seen when these isolates were tested with the higher inoculum in trypticase soy broth (Tables 1 and 2). Also, the larger inoculum did not influence the activity of these cephalosporin compounds against *Streptococcus faecalis* isolates when tested in Mueller-Hinton broth (Tables 3 and 4), but the activity of cefoxitin against these isolates was decreased when the lower inoculum was tested in Mueller-Hinton broth as compared with trypticase soy broth (Tables 1 and 3).

The new 1-oxa compound was bactericidal for most of the species of organisms studied and there was a minimal difference between MIC and MBC. Specifically, the MBC of the 1-oxa compound was only twofold higher than the MIC for both beta-lactamase and non beta-lactamase producing *Staphylococcus aureus* isolates at inocula of 10^5 and 10^7 but only in TSB. In fact, the new compound was more active (lower MIC and MBC) against beta-lactamase producing strains of *S. aureus* in MHB at inocula of either 10^5 or 10^7 organisms. Furthermore, except for *S. faecalis* isolates, MBCs were the same as MICs for 130/135 (96 percent) and 131/135 (97

TABLE 2

Comparative In Vitro Activity of 1-Oxa Cephalosporin and Other Antibiotics in Trypticase Soy Broth with an Inoculum of 1×10^7 cfu/ml

Organism (No. of strains)	Drug	Range of MIC (ug/ml)	50% MIC (ug/ml)	90% MIC (ug/ml)
Pseudomonas sp. (15)	Cephalothin	>128	>128	>128
	Cefamandole	>128	>128	>128
	Cefoxitin	>128	>128	>128
	1-Oxa	8.0–128	16	128
Klebsiella sp. (15)	Cephalothin	8.0–64	16	64
	Cefamandole	4.0–128	16	32
	Cefoxitin	2.0->128	8.0	>128
	1-Oxa	0.12->128	1.0	>128
Serratia sp. (15)	Cephalothin	32.0->128	>128	>128
	Cefamandole	128->128	>128	>128
	Cefoxitin	64.0->128	128	>128
	1-Oxa	0.25->128	8.0	>128
Enterobacter sp. (15)	Cephalothin	>128	>128	>128
	Cefamandole	128->128	>128	>128
	Cefoxitin	128->128	>128	>128
	1-Oxa	0.5->128	16.0	>128
Proteus sp. (15) (Indole neg.)	Cephalothin	8.0->128	32	>128
	Cefamandole	2.0->128	128	>128
	Cefoxitin	4.0->128	>128	>128
	1-Oxa	4.0->128	>128	>128
Proteus sp. (15) (Indole pos.)	Cephalothin	>128	>128	>128
	Cefamandole	>128	>128	>128
	Cefoxitin	4.0->128	128	>128
	1-Oxa	0.2->128	8.0	8.0
S. aureus sp. (15) (Penicillin sensitive)	Cephalothin	<0.12–16	0.25	4.0
	Cefamandole	0.25–4.0	0.5	2.0
	Cefoxitin	2.0–32	2.0	4.0
	1-Oxa	4.0–8.0	8.0	8.0
S. aureus sp. (15) (Penicillin resistant)	Cephalothin	≤0.12–16	<0.12	0.25
	Cefamandole	<0.12–4	0.25	0.5
	Cefoxitin	2.0–32	2.0	2.0
	1-Oxa	4.0->128	4.0	8.0
E. coli (15)	Cephalothin	64.0->128	128	>128
	Cefamandole	2.0->128	2.0	>128
	Cefoxitin	4.0->128	8.0	>128
	1-Oxa	<0.12->128	1.0	>128
Enterococci (15)	Cephalothin	8.0–64.0	32	64
	Cefamandole	16.0–32	32	32
	Cefoxitin	>128	>128	>128
	1-Oxa	>128	>128	>128

TABLE 3
Comparative In Vitro Activity of 1-Oxa Cephalosporin and Other Antibiotics in
Mueller-Hinton Broth with an Inoculum of 1×10^5 cfu/ml

Organism (No. of strains)	Drug	Range of MIC (ug/ml)	50% MIC (ug/ml)	90% MIC (ug/ml)
Pseudomonas sp. (15)	Cephalothin	>128	>128	>128
	Cefamandole	>128	>128	>128
	Cefoxitin	>128	>128	>128
	1-Oxa	4.0–128	8.0	128
Klebsiella sp. (15)	Cephalothin	2.0–16	4.0	16
	Cefamandole	0.5–32	1.0	4.0
	Cefoxitin	4.0->128	4.0	64
	1-Oxa	<0.1	<0.1	<0.1
Serratia sp. (15)	Cephalothin	8.0->128	>128	>128
	Cefamandole	1.0->128	16.0	>128
	Cefoxitin	4.0->128	16.0	64
	1-Oxa	<0.1–16.0	0.25	4.0
Enterobacter sp. (15)	Cephalothin	32–≥128	>128	>128
	Cefamandole	1.0->128	8.0	>128
	Cefoxitin	128->128	>128	>128
	1-Oxa	<0.1–8.0	<0.1	2.0
Proteus sp. (15) (Indole neg.)	Cephalothin	2.0->128	8.0	>128
	Cefamandole	0.5->128	1.0	128
	Cefoxitin	4.0->128	4.0	8.0
	1-Oxa	≤0.1	<0.1	<0.1
Proteus sp. (15) (Indole pos.)	Cephalothin	>128	>128	128
	Cefamandole	0.5->128	8.0	128
	Cefoxitin	4.0->128	16.0	64
	1-Oxa	<0.1	<0.1	<0.1
S. aureus sp. (15) (Penicillin sensitive)	Cephalothin	<0.1–8.0	0.25	0.5
	Cefamandole	<0.1–2.0	0.25	1.0
	Cefoxitin	4.0–8.0	4.0	8.0
	1-Oxa	<0.2–16	8.0	16
S. aureus sp. (15) (Penicillin resistant)	Cephalothin	4.0	4.0	4.0
	Cefamandole	<0.1–0.25	<0.1	0.2
	Cefoxitin	1.0–2.0	1.0	2.0
	1-Oxa	<0.1	<0.1	<0.1
E. coli (15)	Cephalothin	4.0->128	8.0	32
	Cefamandole	0.5–64	1.0	4.0
	Cefoxitin	2.0–32	4.0	32
	1-Oxa	<0.1–0.2	<0.1	0.25
Enterococci (15)	Cephalothin	16.0–32	32	32
	Cefamandole	16.0–32	32	32
	Cefoxitin	>128	>128	>128
	1-Oxa	>128	>128	>128

TABLE 4

Comparative in Vitro Activity of 1-Oxa Cephalosporn and Other Antibiotics in
Mueller-Hinton Borth with an Inoculum of 1 × 10^7 cfu/ml

Organism (No. of strains)	Drug	Range of MIC (ug/ml)	50% MIC (ug/ml)	90% MIC (ug/ml)
Pseudomonas sp. (15)	Cephalothin	>128	>128	>128
	Cefamandole	>128	>128	>128
	Cefoxitin	>128	>128	>128
	1-Oxa	16.0->128	128	>128
Klebsiella sp. (15)	Cephalothin	4.0-64	16	64
	Cefamandole	8.0->128	64	>128
	Cefoxitin	32->128	64	>128
	1-Oxa	16.0->128	32	>128
Serratia sp. (15)	Cephalothin	64->128	>128	>128
	Cefamandole	32->128	>128	>128
	Cefoxitin	64-128	64	128
	1-Oxa	16.0->128	128	>128
Enterobacter sp. (15)	Cephalothin	>128	>128	>128
	Cefamandole	>128	>128	>128
	Cefoxitin	64->128	>128	>128
	1-Oxa	8.0-128	32	128
Proteus sp. (15) (Indole neg.)	Cephalothin	8.0->128	64	>128
	Cefamandole	64->128	>128	>128
	Cefoxitin	32->128	64	64
	1-Oxa	32-128	64	128
Proteus sp. (15) (Indole pos.)	Cephalothin	>128	>128	>128
	Cefamandole	64->128	>128	>128
	Cefoxitin	16.0->128	64	>128
	1-Oxa	0.2->128	32	>128
S. aureus sp. (15) (Penicillin sensitive)	Cephalothin	<0.1->128	0.25	32
	Cefamandole	<0.1-32.0	0.5	32
	Cefoxitin	2.0-64	16	64
	1-Oxa	8.0-16.0	8	16
S. aureus sp. (15) (Penicillin resistant)	Cephalothin	4.0-8.0	4.0	8.0
	Cefamandole	0.25-1.0	0.5	1.0
	Cefoxitin	1.0-2.0	2.0	2.0
	1-Oxa	<0.1-0.25	<0.1	0.25
E. coli (15)	Cephalothin	32->128	32	>128
	Cefamandole	1.0->128	4.0	>128
	Cefoxitin	32-128	64	128
	1-Oxa	1.0->128	64	>128
Enterococci (15)	Cephalothin	16.0-32	32	32
	Cefamandole	32-64	32	64
	Cefoxitin	>128	>128	>128
	1-Oxa	>128	>128	>128

percent) of isolates tested at an inoculum of 10^5 cfu per ml in either TSB or MHB, respectively. Similarly, at the higher inoculum of 10^7 cfu per ml there was no difference in MIC and MBC for 99/135 (73 percent) and 103/135 (76 percent) of isolates tested in either TSB or MHB, respectively. For the remaining isolates, the MBC was only twofold higher than the MIC.

DISCUSSION

The introduction of the "second generation" cephalosporins, cefamandole and cefoxitin, resulted in a significant increase in the antibacterial spectrum of the older "first generation" cephalosporins against many beta-lactamase-producing *Enterobacteriaceae* and *Bacteroides* isolates [3]. The new 1-oxa cephalosporin compound evaluated in the present study represents one of a new group of compounds which can be referred to as "third generation" cephalosporins because these newer compounds further enlarge the spectrum of antibacterial activity of the general class of cephalosporin antibiotics to include isolates of *Pseudomonas aeruginosa*. The 1-oxa cephalosporin compound in the present report has *in vitro* activity similar to another new "third generation" cephalosporin, cefotaxime (HR-756), against *Enterobacteriaceae* and has been shown to be twice as active as cefotaxime against *Pseudomonas aeruginosa* and *Bacteroides fragilis* subspecies *fragilis* [2,3]. Although we did not compare the *in vitro* activity of this new 1-oxa compound to that of cefotaxime, the results of the present study indicate that this new 1-oxa cephalosporin has a broad spectrum of activity against a variety of clinical isolates including gram-negative bacilli as well as staphylococci. It was the most active drug tested against *Klebsiella, Serratia, Enterobacter*, indole negative and positive *Proteus* species, and *E. coli*. Most of these isolates were inhibited by concentrations as low as 0.5 ug/ml of this new compound. Furthermore, 50 percent of the clinical isolates of *Pseudomonas* species were inhibited by concentrations of 16 ug/ml of this compound and, although less active than currently available aminoglycosides, this compound has the potential to be clinically effective in the treatment of infections caused by these bacteria.

The new compound was less active against both beta-lactamase and non beta-lactamase producing staphylococcal isolates than the older cephalosporins, cephalothin and cefamandole, which were the most effective agents tested in this study. However, the 1-oxa compound was effective against all staphylococcal isolates tested at concentrations of 8.0 ug/ml although it had no appreciable activity against *Streptococcus faecalis* isolates.

The *in vitro* activity of this new compound against *Enterobacteriaceae*, as well as that of the other cephalosporins tested in the present study, was influenced by inoculum size in both trypticase soy and Mueller-Hinton broth. This decrease in activity of the 1-oxa compound was also observed against isolates of *Pseudomonas species* in Mueller-Hinton but not in trypticase soy broth. The clinical significance, if any, of these latter observations requires further study.

The results of the present study indicate that this new 1-oxa cephalosporin has a broad spectrum of *in vitro* antibacterial activity which, except for staphylococcal isolates, far exceeds the activity of older cephalosporins, including those agents—particularly cefoxitin and cefamandole—which recently became available for general clinical use. Furthermore, this new compound compares favorably with other antimicrobial agents, including aminoglycosides and antipseudomonal penicillins, against aerobic gram-negative enteric bacilli. Thus, this new antibiotic offers great

promise as a broad-spectrum antibacterial agent and warrants properly controlled future trials to determine its clinical efficacy in man.

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REFERENCES

1. Stapley EO, Birnbaum J, Miller KA, et al: Cefoxitin and cephamycins: microbiological studies. Rev Infect Dis 1:73–89, 1979
2. Neu HC, Aswapokee N, Aswapokee P, et al: HR 756, a new cephalosporin active against gram-positive and gram-negative aerobic and anaerobic bacteria. Antimicrob Ag Chemother 15:273–281, 1979
3. Neu HC, Aswapokee N, Fu K, et al: Antibacterial activity of a new 1-oxa cephalosporin compared with that of other B-Lactam Compounds. Antimicrob Ag Chemother 16:141–149, 1979
4. Bauer AW, Kirby WM, Sherrir JC, et al: Antibiotic susceptibility testing by a standard single dose method. Am J Clin Patho 45:493–496, 1966
5. Lyons RW, Andriole VT: Cephalexin: clinical and laboratory studies. Yale J Biol Med 44:187–198, 1971
6. Valenti AJ, Andriole VT: *In vitro* susceptibility of selected bacteria to cefaclor. Yale J Biol Med 50:465–469, 1977