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Antibacterial Activity of a New 1-Oxa Cephalosporin Compared with That of Other β-Lactam Compounds

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The in vitro activity of (6R,7R)-7-{[carboxy(4-hydroxyphenyl)acetyl]amino}-7-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-oxa-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic acid was tested against isolates of gram-positive and negative bacteria and compared with those of cephalothin, cefuroxime, cefamandole, cefoxitin, cefotaxime, and carbenicillin. The compound was less active than the other compounds when tested against *Staphylococcus aureus* and *Staphylococcus epidermidis*. It had equal or slightly less activity than did cefotaxime when tested against members of the *Enterobacteriaceae*, but was 8- to 32-fold more active than the other cephalosporins against the *Enterobacteriaceae*, inhibiting most isolates at concentrations less than 0.5 μg/ml. The compound was twofold more active than cefotaxime and cefoxitin against *Bacteroides*, and it was twofold more active than cefotaxime and fourfold more active than carbenicillin against *Pseudomonas aeruginosa*. In vitro activity did not correlate with either the presence or type of β-lactamase in either *Enterobacteriaceae* or *Pseudomonas*. The compound showed minimal synergy when combined with aminoglycosides or carbenicillin.

There have been many new cephalosporin antibiotics developed in the past few years. Several of these agents, after extensive in vitro evaluation and clinical investigation, have become available commercially. Cefamandole, cefuroxime, and the cefamycin, cefoxitin, are agents which have significantly enlarged the antibacterial spectrum of the older agents such as cephalothin and cefazolin (2, 4-9). These agents, although they inhibit many strains of β-lactamase-producing *Enterobacteriaceae* and many isolates of *Bacteroides fragilis*, have not inhibited *Pseudomonas aeruginosa*, which has become an increasingly important hospital pathogen. Cefotaxime (HR 756) has been shown by a number of investigators to inhibit gram-positive and -negative aerobic and anaerobic bacteria at concentrations much lower than those required by other agents (1, 3, 8). The development of the oxa-cephalosporins has offered another type of compound which provides an in vitro activity equivalent to that of cefotaxime. For this reason we compared the in vitro activity of (6R,7R)-7-[[carboxy(4-hydroxyphenyl)-acetyl]amino]-7-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio] methyl] -8-oxo-5-oxa-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (Fig. 1) with the in vitro activities of cephalothin, cefamandole, cefoxitin, cefotaxime, and certain other β-lactam compounds.

MATERIALS AND METHODS

Bacterial isolates utilized in the experiments were obtained from clinical specimens submitted to the diagnostic microbiology laboratory of the Columbia Presbyterian Medical Center, New York City. Organisms that were resistant to β-lactam antibiotics and to aminoglycoside antibiotics and had been stored frozen were included in every species to provide a more realistic evaluation of the activity of this compound against multiresistant species.

The compound, hereafter referred to as the "1-oxa cephalosporin," was provided by Eli Lilly and Co. All other antibiotics were gifts of their respective manufacturers. Solutions of antibiotics were prepared fresh daily. Susceptibility studies, unless specified, were performed using agar which contained a twofold dilution of antibiotic. Organisms were delivered with a replicating device which delivered a spot amount of broth containing 10^5 colony-forming units (CFU). Mueller-Hinton agar (BBL Microbiology Systems) was used. Plates were incubated at 35°C for 18 h, and the minimal inhibitory concentration (MIC) was taken as that concentration which showed no visible growth or less than five colonies. Minimal bactericidal concentration (MBC) was determined by use of broth dilution. An inoculum of 10^5 CFU in 1 ml was used, and 0.01 ml from clear tubes was plated on blood agar. The concentration which failed to yield growth or less than five colonies was taken as the MBC. In vitro activity of the 1-oxa compound against *Streptococcus pneumoniae*, *Haemophilus*, *Neisseria*, and other fastidious species was determined using chocolate Mueller-Hinton agar. Anaerobic activity was determined using

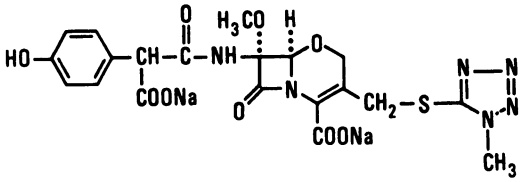

FIG. 1. *Chemical structure of 1-oxa cephalosporin, (6R,7R)-7-[[carboxy(4-hydroxyphenyl)acetyl]amino]-7-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)-thio]-methyl]-8-oxo-5-oxa-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.*

Mueller-Hinton agar supplemented with 5% sheep blood and vitamin K. Anaerobic incubations were for 48 h at 35°C in a Gas-Pak jar.

Killing curves were performed in Mueller-Hinton broth (BBL Microbiology Systems) using a fresh dilution of organisms from an overnight incubation. Samples were taken at selected intervals, immediately diluted in broth, and plated at several dilutions on Mueller-Hinton agar. After overnight incubation the CFU were counted.

Synergy studies were performed on agar using serial twofold dilutions of both agents as previously published (2).

β-Lactamase activity of all isolates was determined by use of the Glaxo chromogenic cephalosporin and with a microiodometric method (6, 10), and enzymes were classified by the method of Sykes and Matthew (11). Organisms were induced to produce β-lactamases by incubation of a fresh overnight culture with either methicillin (5 μg/ml) or cephalothin (25 μg/ml).

Protein binding was determined by the agar diffusion method (8).

RESULTS

The comparative in vitro activities of the 1-oxa cephalosporin and other agents is shown in Table 1. The compound was less active than cephalothin, cefuroxime, or cefamandole against *Staphylococcus aureus* and *Staphylococcus epidermidis*, and failed to inhibit methicillin-resistant *Staphylococcus epidermidis* strains. The compound was several-fold less active than cefotaxime against staphylococci. Higher concentrations of the 1-oxa compound than those of any of the other cephalosporins were required to inhibit *Streptococcus viridans* and *Streptococcus pyogenes*. *Streptococcus agalactiae* showed a bimodal distribution of susceptibility to the 1-oxa compound, which overall was less active than other agents. The compound was manyfold less active against *Streptococcus pneumoniae* than were other cephalosporins or ampicillin, and it had poor inhibitory activity against *Streptococcus faecalis*, similar to older cephalosporins.

The 1-oxa compound inhibited members of the *Enterobacteriaceae* at lower concentrations than did the older cephalosporins or the newer ones cefamandole, cefuroxime, and cefoxitin, but it was less active against many isolates than cefotaxime. For example, 87% of the *Escherichia coli* isolates tested were inhibited by 0.05 μg of cefotaxime per ml, whereas the 1-oxa compound inhibited 53% at this concentration. At 0.05 μg/ml cefotaxime inhibited 65% of *Klebsiella* isolates, and the 1-oxa compound at 0.05 μg/ml inhibited 35% of *Klebsiella* isolates. Similar results were found for *Enterobacter aerogenes*, *Enterobacter cloacae*, *Citrobacter freundii*, and *Citrobacter diversus*. The in vitro activity of the 1-oxa compound and cefotaxime were quite similar against *Proteus rettgeri*, *Proteus vulgaris*, *Proteus mirabilis*, *Morganella morganii*, and *Salmonella* species, including *Salmonella typhi*. The 1-oxa compound was the most active compound tested against *Providencia*, *Shigella*, and *Serratia*. It was twofold more active than cefotaxime and fourfold more active than carbenicillin against *P. aeruginosa*. It was similar in activity to cefsulodin and ticarcillin but less active than piperacillin against these isolates of *P. aeruginosa*.

The activity of this compound against anaerobic species was tested using *B. fragilis* subsp. *fragilis* and other *Bacteroides*. The 1-oxa compound was more active than the other agents. It inhibited 50% of highly resistant *B. fragilis* at concentrations of 6.2 μg/ml, similar to the concentration required with cefoxitin. The compound was several-fold more active than cefotaxime against *Bacteroides*. Other anaerobic species were tested, but are not shown in the table because comparative activity was not determined. The 1-oxa compound inhibited the species of *Clostridium* tested (five isolates) at concentrations less than 1 μg/ml; *Fusobacteria* (four isolates) were inhibited at concentrations of 1 to 12 μg/ml. The concentrations required to inhibit peptostreptococci and peptococci were 6 to 50 μg/ml.

β-Lactamase-producing *Salmonella typhi*, *Neisseria gonorrhoeae*, and *Haemophilus influenzae* were inhibited by the 1-oxa compound, but the concentrations were several-fold greater than those we had found earlier for the same isolates tested with cefuroxime, cefamandole, and cefotaxime.

Effect of alteration of test conditions. The effect of the growth medium upon MICs was tested for *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *P. aeruginosa*, and *E. cloacae*. The MICs of representative isolates of these species tested in brain heart infusion, Trypticase soy, nutrient, and Columbia broths were within a single dilution of each other in all instances. Comparison of MICs in Mueller-Hinton broth and in Muel-

TABLE 1. *Comparative in vitro activity of 1-oxa cephalosporin and other β-lactam antibiotics*

Organism (no. of strains)	Drug	Range of MIC (μg/ml)	50% MIC (μg/ml)	90% MIC (μg/ml)
Staphylococcus aureus (18)	1-Oxa	3.1–25	6.2	6.2
	Cephalothin	0.1–0.25	0.1	0.2
	Cefamandole	0.25–6.2	0.2	0.8
	Cefuroxime	0.2–25	0.2	0.8
	Cefoxitin	0.4–25	3.1	6.2
	Cefotaxime	0.4–25	1.6	3.1
Staphylococcus epidermidis (16)	1-Oxa	0.4–>100	25	50
	Cephalothin	0.2–25	0.8	1.6
	Cefamandole	0.2–25	0.8	1.6
	Cefoxitin	0.2–25	3.1	6.2
	Cefotaxime	0.2–25	3.1	6.2
	Methicillin	0.8–25	0.8	12.5
Streptococcus pyogenes (15)	1-Oxa	0.8–3.1	0.8	1.6
	Cephalothin	0.05–0.2	0.05	0.2
	Cefuroxime	0.05–0.2	0.1	0.2
	Cefamandole	0.05–0.2	0.1	0.2
	Cefotaxime	0.01–0.2	0.05	0.1
Streptococcus agalactiae (20)	1-Oxa	0.4–3.1	0.8	3.1
	Cephalothin	0.1–0.8	0.1	0.4
	Cefamandole	0.1–0.8	0.1	0.4
	Cefotaxime	0.01–0.2	0.05	0.1
Streptococcus viridans (5)	1-Oxa	0.4–>100	12.5	>100
	Cephalothin	0.05–0.8	0.1	0.4
	Cefotaxime	0.05–0.8	0.1	0.4
Streptococcus faecalis (13)	1-Oxa	3.1–>100	>100	>100
	Cephalothin	12.5–>100	25	50
	Cefamandole	12.5–>100	25	50
	Cefoxitin	25–>100	25	50
	Ampicillin	0.2–3.1	0.8	1.6
	Cefotaxime	1.6–>100	12.5	>100
Streptococcus pneumoniae (10)	1-Oxa	0.2–6.1	1.6	3.1
	Cephalothin	0.1–0.4	0.1	0.2
	Cefamandole	0.1–0.4	0.1	0.2
	Cefotaxime	0.01–0.2	0.01	0.1
Haemophilus influenzae (12)	1-Oxa	0.1–3.1	0.1	1.6
	Cephalothin	0.2–25	0.8	0.8
	Cefamandole	0.2–1.6	0.2	0.4
	Cefoxitin	0.4–3.1	0.2	1.6
	Cefuroxime	0.2–1.6	0.2	0.8
	Ampicillin	0.05–25	0.4	1.6
Neisseria gonorrhoeae (13)	1-Oxa	0.4–1.6	0.1	0.8
	Cephalothin	0.4–12.5	0.8	3.1
	Cefuroxime	0.2–0.8	0.2	0.8
	Cefoxitin	0.2–1.6	0.4	0.8
	Cefotaxime	0.01–0.4	0.01	0.4
Escherichia coli (40)	1-Oxa	0.02–12.5	0.1	0.1
	Cephalothin	1.6–100	6.2	50
	Cefamandole	0.2–6.2	1.6	3.1
	Cefuroxime	1.6–12.5	3.1	6.2
	Cefoxitin	1.6–12.5	3.1	6.2
	Cefotaxime	<0.02–3.1	0.1	0.4
	Carbenicillin	1.6–>400	12.5	>400

TABLE 1.—*Continued*

Organism (no. of strains)	Drug	Range of MIC (μg/ml)	50% MIC (μg/ml)	80% MIC (μg/ml)
Klebsiella pneumoniae (37)	1-Oxa	0.05–12.5	0.1	0.8
	Cephalothin	1.6–100	6.2	50
	Cefamandole	0.4–100	3.1	50
	Cefoxitin	0.8–100	1.6	25
	Cefotaxime	0.02–0.4	0.05	0.4
Enterobacter aerogenes (16)	1-Oxa	0.05–3.1	0.2	3.1
	Cephalothin	6.2–>100	>100	>100
	Cefamandole	0.2–>100	3.1	25
	Cefoxitin	6.2–>100	>100	>100
	Cefotaxime	0.05–>100	0.1	0.4
	Carbenicillin	1.6–>100	6.2	>100
Enterobacter cloacae (18)	1-Oxa	0.05–25	0.2	12.5
	Cephalothin	25–>100	>100	>100
	Cefamandole	0.2–>100	3.1	50
	Cefoxitin	12.5–>100	>100	>100
	Cefotaxime	0.05–0.8	0.1	0.4
	Carbenicillin	3.1–>100	12.5	>100
Proteus mirabilis (34)	1-Oxa	0.05–1.6	0.1	0.1
	Cephalothin	1.6–100	3.1	12.5
	Cefamandole	0.4–100	3.1	25
	Cefoxitin	0.4–25	3.1	12.5
	Cefotaxime	0.02–3.1	0.05	0.1
	Carbenicillin	0.8–>100	1.6	3.1
Proteus vulgaris (11)	1-Oxa	<0.1–25	0.1	3.1
	Cephalothin	>100	>100	>100
	Cefamandole	12.5–>100	>100	>100
	Cefoxitin	11.6–>100	3.1	3.1
	Cefotaxime	12.5–>100	>100	>100
	Carbenicillin	0.8–>100	25	50
Proteus rettgeri (13)	1-Oxa	<0.1–25	0.1	12.5
	Cefamandole	0.2–>100	25	>100
	Cefuroxime	0.8–>100	50	>100
	Cefoxitin	1.6–>100	12.5	>100
	Cefotaxime	0.01–1.6	0.05	1.6
	Carbenicillin	0.8–>100	>100	>100
Morganella morganii (15)	1-Oxa	0.1–0.2	0.1	0.1
	Cefamandole	1.6–25	12.5	25
	Cefuroxime	0.8–25	6.2	12.5
	Cefoxitin	6.2–25	12.5	12.5
	Cefotaxime	0.01–1.6	0.02	1.6
	Carbenicillin	0.4–12.5	0.8	3.1
Providencia (10)	1-Oxa	0.05–0.1	0.1	0.1
	Cefamandole	0.8–>100	25	>100
	Cefuroxime	1.6–>100	25	>100
	Cefoxitin	1.6–>100	6.2	12.5
	Cefotaxime	0.05–3.1	0.4	0.8
	Carbenicillin	3.1–100	12.5	>100
Citrobacter (10)	1-Oxa	<0.02–0.2	0.1	0.2
	Cephalothin	3.1–>100	6.2	>100
	Cefamandole	0.2–6.2	0.8	6.2
	Cefuroxime	0.8–6.2	3.1	6.2
	Cefoxitin	1.6–>100	12.5	>100
	Cefotaxime	0.02–0.1	0.1	0.1
	Carbenicillin	0.8–>100	3.1	>100

TABLE 1.—*Continued*

Organism (no. of strains)	Drug	Range of MIC (μg/ml)	50% MIC (μg/ml)	80% MIC (μg/ml)
Serratia (34)	1-Oxa	0.05–50	12.5	50
	Cefamandole	50–>100	>100	>100
	Cefuroxime	50–>100	>100	>100
	Cefoxitin	12.5–>100	>100	>100
	Cefotaxime	0.1–50	12.5	50
Salmonella (17)	1-Oxa	0.02–1.6	0.1	1.6
	Cephalothin	1.6–25	3.1	6.1
	Cefamandole	0.2–25	0.8	12.5
	Cefuroxime	3.1–12.5	3.1	6.2
	Cefoxitin	1.6–6.2	1.6	3.1
	Cefotaxime	0.02–0.2	0.1	0.1
Shigella (18)	1-Oxa	0.05–0.4	0.1	0.2
	Cephalothin	1.6–>100	3.1	25
	Cefamandole	0.2–>100	0.8	25
	Cefoxitin	1.6–>100	3.1	25
	Cefotaxime	0.05–1.6	0.1	0.8
Acinetobacter (11)	1-Oxa	1.6–>100	50	>100
	Cephalothin	25–>100	>100	>100
	Cefamandole	3.1–>100	>100	>100
	Cefuroxime	0.8–>100	>100	>100
	Cefoxitin	1.6–>100	>100	>100
	Cefotaxime	1.6–>100	50	>100
	Carbenicillin	1.6–>100	25	>100
Bacteroides fragilis subsp. *fragilis* (11)	1-Oxa	0.05–100	6.2	50
	Cefamandole	25–>100	>100	>100
	Cefoxitin	3.1–>100	6.2	>100
	Cefotaxime	12.5–>100	50	>100
	Carbenicillin	25–>100	25	>100
Bacteroides sp. (21)	1-Oxa	0.05–>100	0.05	>100
	Cephalothin	25–>100	>100	>100
	Cefamandole	12.5–>100	>100	>100
	Cefoxitin	6.2–>100	6.2	50
	Cefotaxime	3.1–>100	25	>100
	Carbenicillin	12.5–>100	50	>100
Pseudomonas aeruginosa (79)	1-Oxa	6.2–>100	12.5	>100
	Cefoxitin	>100	>100	>100
	Cefotaxime	0.4–>100	25	>100
	Carbenicillin	25–>100	50	>100
	Ticarcillin	0.8–>100	25	100
	Piperacillin	0.4–>100	6.2	25
	Cefsulodin	0.8–>100	3.1	50

ler-Hinton agar revealed no differences for *E. coli*, *K. pneumoniae*, *P. vulgaris*, *Providencia*, *Serratia*, *E. cloacae*, *E. aerogenes*, *P. aeruginosa*, and *Staphylococcus aureus*.

Table 2 illustrates the effect of inoculum size on both MIC and MBC values of isolates, all of which contained β-lactamases. At a low inoculum, 10^3 CFU, there was minimal difference between MIC and MBC for *E. coli* and *E. cloacae*. There was a definite increase in MICs and MBCs as the inoculum was increased to 10^7 CFU. The two *Klebsiella* isolates, selected because of high-level β-lactamase production, both showed a large difference between the MIC and MBC at the three inocula tested. One organism had the same MIC at an inoculum of 10^3 and 10^7 CFU, whereas the other showed an increase of 16-fold in MICs. One *P. aeruginosa* with an MIC of >100 μg/ml was resistant at all inocula, whereas the other was resistant at 10^7 CFU but was not resistant at 10^3 or 10^5 CFU. The MICs and MBCs were determined for a number of

TABLE 2. *Effect of inoculum size on the MIC and MBC*

Organism	10^7 CFU		10^5 CFU		10^3 CFU	
	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)
E. cloacae	6.2	6.2	6.2	6.2	3.1	3.1
E. cloacae	12.5	50	0.8	50	0.1	0.4
E. coli	3.1	50	0.6	6.2	0.8	0.8
E. coli	3.1	12.5	0.05	0.2	0.05	0.2
K. pneumoniae	6.2	100	0.4	50	0.4	50
K. pneumoniae	12.5	100	12.5	100	12.5	50
P. aeruginosa	>100	>100	>100	>100	>100	>100
P. aeruginosa	>100	>100	25	>100	25	25

other organisms using an inoculum of 10^5 CFU. There was an 8- to 16-fold difference between the MIC and MBC for seven *H. influenzae* tested. The increase in MBC over MIC for *P. mirabilis*, indole-positive *Proteus*, and *Providencia* was only two- to fourfold.

Killing curve studies were performed with the 1-oxa compound, cefotaxime, and cephalothin or carbenicillin. The 1-oxa compound and cefotaxime, as Fig. 2 and 3 show, had similar activities against *E. coli* and *Klebsiella* with a 5-log reduction in CFU in 24 h. However, when tested against *P. aeruginosa*, regrowth occurred with both the 1-oxa compound and with carbenicillin. The same was seen when the compounds were tested against a *Serratia* susceptible to both cefotaxime and 1-oxa compound.

Table 3 shows a direct comparison of the 1-oxa compound and cefotaxime with other agents against isolates resistant to cephalothin or carbenicillin. The two agents differed in activity to a minor degree, but both inhibited organisms resistant to cefamandole, cefuroxime, cefoxitin, and carbenicillin at concentrations usually below 6 μg/ml.

We attempted to correlate the presence and type of β-lactamase with MICs. As Fig. 4 demonstrates, there was no correlation of the presence of absence of β-lactamase with MIC values. MICs of 0.1 μg/ml were found in organisms in which a β-lactamase could not be demonstrated and in species that contained constitutive β-lactamase, often primarily with cephalosporinase activity or induced enzymes. Similarly, organisms with or without a β-lactamase had MICs of 6.2 μg/ml. This was particularly true of *Staphylococcus aureus*, *Serratia*, and *Bacteroides*.

Synergy studies. The 1-oxa compound was combined with gentamicin and tested against *Pseudomonas* isolates that were susceptible or resistant to both or either compound, and it also

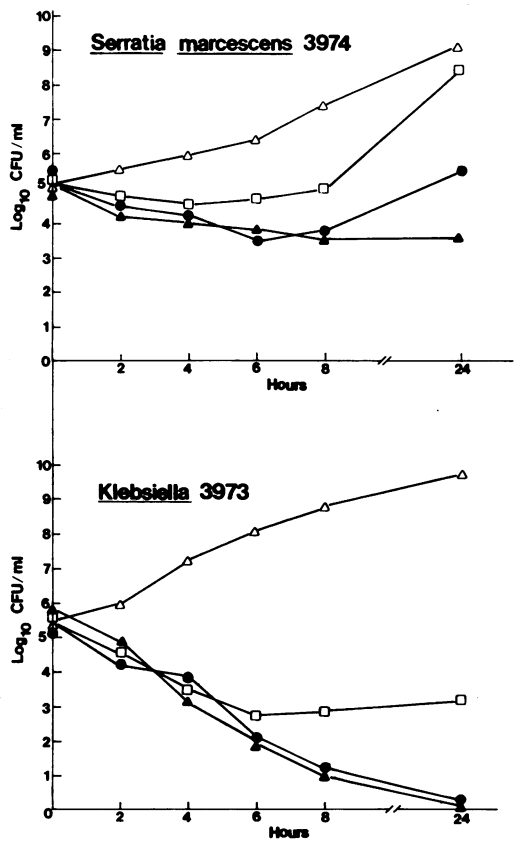

FIG. 2. *Time-kill curves against Klebsiella and Serratia with 1-oxa cephalosporin (▲), compared with cephalothin (□), cefotaxime (●), and control (△) at the concentration of two times the MICs of each antibiotic. The MIC of 1-oxa cephalosporin against Klebsiella was 0.8 μg/ml, that of cefotaxime was 0.05 μg/ml, and that of cephalothin was 100 μg/ml. Against Serratia the MICs were 800 μg/ml for cephalothin, 12.5 μg/ml for 1-oxa cephalosporin, and 200 μg/ml for cefotaxime.*

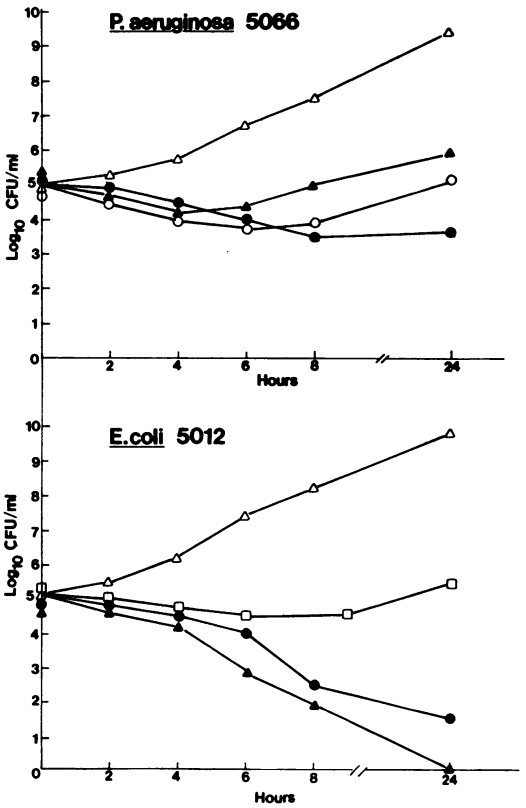

FIG. 3. *Time-kill curves against P. aeruginosa and E. coli with 1-oxa cephalosporin compared with cephalothin, cefotaxime, and carbenicillin at a concentration of two times the MICs of each antibiotic. Symbols are the same as in Fig. 2 except carbenicillin* (○). *The MIC of 1-oxa cephalosporin against P. aeruginosa was 6.3 μg/ml, that of carbenicillin was 100 μg/ml, and that of cefotaxime was 12.5 μg/ml. Against E. coli the MICs were 0.2 μg/ml for 1-oxa cephalosporin, 6.3 μg/ml for cephalothin, and 0.1 μg/ml for cefotaxime.*

was combined with amikacin and tested against isolates resistant to the 1-oxa compound. Of the 21 isolates studied, only 19% were inhibited synergistically by the combination of the 1-oxa compound and gentamicin, and no complete synergy could be demonstrated for the 1-oxa cephalosporin-plus-amikacin combination.

Since we had earlier seen antagonism when certain penicillins and cephalosporins were combined, we tested the combination of the 1-oxa compound and carbenicillin against a number of different species (Table 4). Of the 31 isolates tested, only 10% showed complete synergy and 22% showed partial synergy. With one *Staphylococcus epidermidis* strain the combination was antagonistic. Demonstration of synergy was not related to whether an organism was susceptible or resistant to carbenicillin. No organism resistant to both compounds was inhibited synergistically.

Protein binding. The protein binding of the 1-oxa cephalosporin determined by the agar diffusion method was 11%.

DISCUSSION

The introduction of cefamandole, cefuroxime, and cefoxitin significantly increased the antibacterial spectrum of the older cephalosporins against many β-lactamase-producing *Enterobacteriaceae* and *Bacteroides* (4, 5, 9). Cefotaxime further enlarged the spectrum of cephalosporins to include *P. aeruginosa* (1, 3, 8). The 1-oxa cephalosporin compound discussed in this manuscript has an in vitro activity similar to that of cefotaxime against *Enterobacteriaceae*, inhibiting isolates resistant to cefamandole, cefuroxime, and cefoxitin. In general, this agent was twofold less active than cefotaxime against members of the *Enterobacteriaceae*, but it inhibited the majority of isolates at less than 0.8 μg/ml. Furthermore, the compound was twofold more active than cefotaxime against *P. aeruginosa* and *B. fragilis* subsp. *fragilis*.

Although this compound showed less inhibitory activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* than did many other cephalosporins or the semisynthetic antistaphylococcal penicillins, it was not less active than cefoxitin, which has proved to be effective in the treatment of staphylococcal infections (7).

The presence or absence of plasmid or chromosomal β-lactamases did not affect the in vitro activity of the compound, but there was an effect of inoculum size upon both MIC and MBC in some species. The 1-oxa compound inhibited bacteria resistant to cefamandole and cefuroxime by virtue of β-lactamases which hydrolyze these compounds (Fu and Neu, manuscript in preparation). Although the killing activity of this cephalosporin was similar to that of other agents, the regrowth of *P. aeruginosa* will require further study.

The 1-oxa compound when combined with aminoglycosides rarely showed a synergistic action. It also was not synergistic with carbenicillin, nor did it act antagonistically.

These in vitro results indicate that this agent has the potential of cefotaxime and far exceeds the activity of the agents which recently became available for general clinical use, namely, cefuroxime, cefamandole, and cefoxitin. All of these latter have been used with success in various

TABLE 3. *Comparative activity of newer cephalosporins against cephalothin-resistant, β-lactamase-containing bacteria*

Organism	MIC (μg/ml) of drug:						
	1-oxa	Cefotaxime	Cephalothin	Cefoxitin	Cefamandole	Cefuroxime	Carbenicillin
E. coli	0.1	0.05	50	1.6	100	3.1	>400
E. coli	0.4	0.1	25	12.5	3.1	12.5	25
E. aerogenes	0.8	0.02	>400	>400	>400	>400	>400
E. aerogenes	0.4	3.1	>400	>400	50	100	25
E. cloacae	6.2	0.1	>400	>400	>400	12.5	>400
E. cloacae	0.2	0.05	>400	>400	3.1	3.1	50
Salmonella typhimurium	0.1	0.05	25	1.6	12.5	3.1	>400
Shigella sonnei	0.2	1.6	>200	100	50	12.5	100
P. mirabilis	0.1	3.1	>400	>400	>400	>400	>400
P. vulgaris	0.8	0.1	>400	3.1	>200	>200	25
P. rettgeri	0.1	1.6	>400	100	200	100	>400
K. pneumoniae	0.8	0.05	100	25	50	25	>400
K. pneumoniae	6.2	0.4	50	100	25	25	>400
Serratia marcescens	12.5	25	>400	>400	>400	>400	>400
P. aeruginosa	6.2	25	>400	>400	>400	>400	>200
P. aeruginosa	12.5	50	>400	>400	>400	>400	100
B. fragilis	1.6	25	>400	12.5	>400	>400	>400
B. fragilis	3.2	50	>400	25	>400	>400	>400

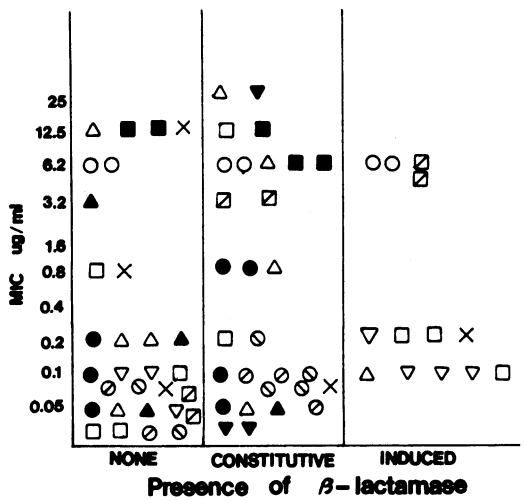

FIG. 4. Relation of the activity of 1-oxa cephalosporin to the presence of β-lactamases. *S. aureus* (○); *E. coli* (●); *E. cloacae* (△); *E. aerogenes* (▲); *M. morganii* (▽); *Klebsiella* (□); *Serratia* (■); *Salmonella* (○); *Shigella* (○); *P. vulgaris* (×); *P. rettgeri* (▼); *and B. fragilis* (□).

clinical settings. It will take a number of years to determine if the increased in vitro activity of agents such as this compound will offer increased benefit clinically.

TABLE 4. *Synergy of carbenicillin and the 1-oxa cephalosporin*

Organism	No. of strains tested	No. of strains showing:		
		Synergy	Partial synergy	Antagonism
E. coli	4		2	
Klebsiella	3	1	1	
Enterobacter	3	2		
Citrobacter	3			
Serratia	3		1	
Salmonella	2			
Shigella	1			
Proteus, indole-positive	3		1	
Pseudomonas	4		2	
S. aureus	2			
S. epidermidis	3			1

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