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HR 756, a New Cephalosporin Active Against Gram-Positive and Gram-Negative Aerobic and Anaerobic Bacteria

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The in vitro activity of HR 756, 7-[2-(2-amino-4-thiazolyl)-2-(Z)-(methoximino)acetamido] cephalosporanic acid, was investigated against 659 isolates. HR 756 inhibited *Neisseria* and *Haemophilus* species at concentrations similar to those needed with ampicillin. It inhibited β-lactamase-producing *N. gonorrhoeae* and *H. influenzae*. HR 756 was the most active compound tested against members of the *Enterobacteriaceae*, inhibiting most isolates of *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella, Enterobacter*, and *Shigella* at concentrations of less than 0.1 μg/ml. It was twice as active as carbenicillin against *Pseudomonas aeruginosa* and inhibited *Bacteroides fragilis* as well as cefoxitin. HR 756 killed *E. coli, Staphylococcus aureus,* and *P. aeruginosa* at rates similar to other β-lactam antibiotics.

The types of infections in which cephalosporins or cephamycins have been utilized have been infections in which several bacteria have been present or infections in patients with host defenses compromised by virtue of a long hospital stay and thus caused by resistant *Escherichia coli* or *Klebsiella pneumoniae* or by organisms such as *Enterobacter, Serratia,* or *Pseudomonas*. These latter organisms often have been resistant both to older cephalosporins and even to the new ones. Recently a cephalosporin, HR 756, 7-[2-(2-amino-4-thiazolyl)-2-(Z)-(methoximino)acetamido] cephalosporanic acid, has been reported to be active against *Pseudomonas* (5, 6). We have shown that this agent is resistant to β-lactamases (5) and so have undertaken an extensive evaluation of this agent to compare its activity to other β-lactam compounds, both penicillins and cephalosporins.

MATERIALS AND METHODS

HR 756 was a gift of Hoechst-Roussel. All other antibiotics were provided by their respective manufacturers. Fresh dilutions of the compounds were prepared daily in sterile medium or distilled water. Bacterial strains were recent blood, sputum, or urine isolates from patients hospitalized within the past 2 years at the Columbia-Presbyterian Medical Center, New York, N.Y. As specifically noted, isolates saved frozen over the past 10 years were used in some experiments because they were known to contain enzymes mediating resistance via β-lactamases or aminoglycoside-inactivating enzymes.

Susceptibility tests. Antimicrobial activity was measured by agar dilution or broth methods as specified. Mueller-Hinton (MH) agar or broth was used unless otherwise specified. An inoculum of 10^5 colony-forming units (CFU) prepared by dilution of a fresh overnight culture was used. Plates or tubes were incubated at 35°C for 18 h. Minimal inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic that inhibited development of visible growth on agar or in broth. Minimal bactericidal concentration (MBC) was determined by plating 0.1 ml from clear broth tubes to blood agar. The MBC was the concentration at which there were fewer than five colonies after 24 h of incubation at 35°C. Susceptibility of streptococci was determined with MH agar supplemented with 5% sheep blood. Susceptibility of *Neisseria* species and *Haemophilus* species was determined on chocolate MH agar with assays run in the presence of CO_2. Tube dilutions for these species were performed with Levinthal broth. Anaerobic susceptibility was determined with MH agar supplemented with sheep blood and vitamin K. Incubation of anaerobic cultures was for 48 h in GasPak jars (Baltimore Biological Laboratory).

Killing curves were derived from experiments in broth with exponentially growing cells. Antibiotic was present at the concentration denoted in the particular experiment. Samples were removed from a gyratory shaker, immediately diluted in broth, and plated on MH agar to determine the CFU present.

Synergy study. Synergy of HR 756 with other antibiotics was determined by the agar dilution, checkerboard isobologram, and killing curve techniques previously described (4). Synergy was considered to be present when there was a fourfold decrease in the MICs or MBCs of both agents or a 2-log decrease in CFU in a killing experiment.

Protein binding. Protein binding was determined by three methods. In the first, HR 756 was dissolved in pooled, normal human serum and centrifuged for 18 h at 124,000 × *g* in an SW50.1 rotor of a Spinco preparative ultracentrifuge. Samples were removed

and assayed for free antibiotic by an agar well plate technique with *E. coli* 3998 (our collection) as the assay organism. This will detect 0.16 μg of antibiotic per ml. In the second, HR 756 dissolved in pooled human serum was dialyzed at 4°C for 6 h against potassium phosphate buffer (pH 7.4, 0.05 M). Dialysate and serum were assayed for antibiotic by the agar well technique. In the third method, the zones of inhibition in the agar well system produced by HR 756 were compared in diluted buffer and serum.

β-Lactamase assays. Both the chromogenic substrate 87/132 (13) and a modified iodometric assay were used. Induction of β-lactamases was with cephalothin (2, 9). β-Lactamases were classified by the Richmond method (15).

RESULTS

Antibacterial activity—comparative studies. The activity of HR 756 was compared with that of other β-lactams, both penicillins and cephalosporins, agents currently in clinical use, as well as recently developed cephalosporins and cephamycins which will shortly become available for clinical use. The results of these comparisons have been summarized in Table 1. HR 756 was less active than cephalothin or cefamandole against *Staphylococcus aureus* but comparable in activity to that of cefoxitin. HR 756 was more active than any of the cephalosporins tested against β-hemolytic streptococci, group A and B, but it was less active than ampicillin against enterococci, such as *Streptococcus faecalis*. HR 756 showed a bimodal activity against enterococci, with half of the isolates inhibited by 6 μg or less per ml. HR 756 and ampicillin had comparable activity against *Streptococcus pneumoniae*. HR 756 was similar in activity to the other cephalosporins against *Neisseria gonorrhoeae* but was less active than ampicillin against one-fourth of the β-lactamase-negative strains. Against *Haemophilus influenzae*, HR 756 had activity similar to that of cefuroxime but was less active than was cefamandole.

HR 756 was the most active β-lactam tested against the members of the *Enterobacteriaceae*. Tested against *E. coli*, 15% of which were cephalothin resistant and 35% of which were carbenicillin resistant, HR 756 inhibited 90% at 0.4 μg/ml and was more active than cefoxitin, cefamandole, or cefuroxime. *K. pneumoniae*, 30% of which were cephalothin resistant, were inhibited by HR 756 at 32- to 64-fold-lower concentrations than those of cefuroxime or cefoxitin. HR 756 was more active than cefamandole, cefuroxime, or carbenicillin against all *Enterobacter*, *E. aerogenes*, *E. cloacae*, and *E. hafnia*. HR 756 was 64-fold more active than cefamandole and cefuroxime, the most active cephalosporins tested, against *Proteus mirabilis* and inhibited all isolates at 1.6 μg/ml compared with concentrations of 12.5 to 200 μg/ml required by the other agents. HR 756 was more active than carbenicillin or cefoxitin against *Proteus morganii*, *Proteus rettgeri*, *Proteus vulgaris*, and *Providencia stuartii*, inhibiting 90% of isolates at concentrations at which only 60 and 40% of isolates were inhibited by the next most active agent. HR 756 was more active than cefamandole or cefoxitin against *Salmonella* species, the majority of which contained β-lactamases. It also was more active than other agents against *Shigella* which contained β-lactamases.

HR 756 was more active than the other agents tested against *Citrobacter* and *Acinetobacter*. The *Serratia* tested all were highly resistant isolates, with plasmids mediating resistance to both β-lactam antibiotics and aminoglycosides. Although HR 756 was the most active agent, the activity against these isolates was poor in contrast to its activity against other bacteria. The pigmented *Serratia* isolates susceptible to many antibiotics were inhibited by 0.2 or 0.4 μg/ml, a concentration 64-fold less than the concentration of the next most active agent, the cephamycin cefoxitin.

HR 756 inhibited *Pseudomonas aeruginosa* at levels below those required for carbenicillin. It was, however, less active than piperacillin or any of the aminoglycosides. At a concentration of 50 μg/ml, HR 756 inhibited 50% of the other *Pseudomonas* species tested, whereas none of the cephalosporins or cephamycins inhibited these organisms at concentrations below 400 μg/ml.

Against anaerobic organisms, particularly *Bacteroides fragilis*, HR 756 was more active than carbenicillin, but less active than cefoxitin, inhibiting 60% of isolates at 25 μg/ml compared with 97% of isolates inhibited by 25 μg of cefoxitin per ml. HR 756 was not more active against non-*fragilis Bacteroides* than was cefoxitin.

The in vitro activity of HR 756 was determined against small numbers of isolates of other organisms. The inhibitory levels were as follows: *H. parainfluenzae*, <0.01 μg/ml (3 isolates); *Haemophilus aphrophilus*, 0.025 μg/ml (1 isolate); *Bacillus subtilis*, 100 μg/ml (1 isolate); *B. cereus*, 100 μg/ml (1 isolate); *Listeria monocytogenes*, 3.1 to 100 μg/ml (10 isolates); *P. multocida*, 0.4 μg/ml (1 isolate); *K. ozenae*, 0.025 μg/ml (2 isolates); *E. hafnia*, 0.05 to 0.8 μg/ml (3 isolates); *E. agglomerans*, <0 .01 to 1.6 μg/ml (3 isolates); *Aeromonas hydrophilia*, 0.025 μg/ml (2 isolates); *Salmonella typhi*, 0.05 μg/ml (2 isolates); *Salmonella arizona*, 0.05 to 0.2 μg/ml (3 isolates); *Pseudomonas stutzeri*, 25 μg/ml (1 isolate); *Clostridium perfringens*, 3.1 μg/ml (1 isolate); peptostreptococci, 200 μg/ml

TABLE 1. *Comparative in vitro activity of HR 756 and other β-lactam antibiotics*

Organism (no. of strains)	Drug	MIC (μg/ml) range	MIC_{50} (μg/ml)	MIC_{90}
S. aureus (32)	HR 756	0.4–3.1	1.6	3.1
	Cephalothin	0.05–0.4	0.1	0.2
	Cefamandole	0.4–0.8	0.4	0.8
	Ampicillin	0.05–100	1.6	100
	Cefuroxime	0.2–1.6	0.2	0.8
S. pyogenes (22)	HR 756	0.01–0.2	0.05	0.2
	Cefuroxime	0.4–6.3	1.56	3.1
	Cephalothin	0.05–0.2	0.05–2	0.2
	Cefamandole	0.05–0.2	0.1	0.2
	Cefuroxime	0.05–0.2	0.1	0.2
S. agalactiae (20)	HR 756	<0.01–0.2	0.05	0.1
	Cephalothin	0.1–0.8	0.1	0.2
	Cefamandole	0.1–0.2	0.1	0.1
	Cefuroxime	0.1–0.2	0.1	0.1
S. pneumoniae (22)	HR 756	<0.01–0.2	<0.01	0.1
	Ampicillin	<0.01–0.1	<0.01	0.1
S. faecalis (30)	HR 756	0.01–200	12.5	200
	Carbenicillin	12.5–50	25	25
	Ampicillin	0.4–3.1	0.8	1.6
	Cephalothin	12.5–100	25	25
	Cefoxitin	25–100	100	100
N. gonorrhoeae (12)	HR 756	<0.01–0.4	<0.01	0.4
	Cephalothin	0.2–2.5	0.8	3.1
	Cefamandole	0.2	0.2	0.8
	Cefuroxime	0.2–0.8	0.2	0.8
	Ampicillin	<0.01–0.1	<0.01	0.1
H. influenzae (16)	HR 756	<0.01–3.1	0.4	0.8
	Cephalothin	0.2–50	0.8	0.8
	Cefamandole	0.2–1.6	0.2	0.4
	Cefuroxime	0.2–1.6	0.2	0.8
	Ampicillin	<0.01–3.1	0.1	1.6
E. coli (30)	HR 756	0.02–50	0.1	0.4
	Cephalothin	1.6–50	6.2	12.5
	Cefoxitin	1.6–12.5	3.1	6.2
	Cefamandole	0.2–100	0.8	3.1
	Cefuroxime	3.1–25	3.1	6.2
	Carbenicillin	3.1–>400	6.2	400
K. pneumoniae (30)	HR 756	<0.01–200	0.05	0.4
	Cephalothin	1.6–100	6.2	50
	Cefoxitin	0.8–>400	3.1	25
	Cefamandole	0.4–100	3.1	50
	Cefuroxime	0.8–>400	3.1	25
	Carbenicillin	50–>400	400	>400
E. aerogenes (21)	HR 756	<0.01–3.1	0.05	0.4
	Carbenicillin	1.6–>400	6.2	200
	Cefoxitin	1.6–>400	400	>400
	Cefamandole	0.2–>400	3.1	25
	Cefuroxime	1.6–>400	6.2	12.5

TABLE 1. *(continued)*

Organism (no. of strains)	Drug	MIC (μg/ml) range	MIC_{50} (μg/ml)	MIC_{90}
E. cloacae (21)	HR 756	0.01–100	0.1	0.8
	Carbenicillin	3.1–400	3.1	100
	Cefoxitin	0.8–>400	100	400
	Cefamandole	0.2–200	3.1	50
	Cefuroxime	1.6–25	6.2	12.5
P. mirabilis (30)	HR 756	<0.01–3.1	<0.01	0.1
	Carbenicillin	0.2–>400	0.8	1.6
	Cefoxitin	0.4–25	3.1	12.5
	Cefamandole	0.4–100	1.6	12.5
	Cefuroxime	0.4–100	1.6	50
P. morganii (14)	HR 756	<0.01–1.6	0.02	1.6
	Carbenicillin	<0.4–12.5	<0.4	3.1
	Cefoxitin	6.2–400	12.5	12.5
	Cefamandole	1.6–100	12.5	25
	Cefuroxime	0.8–25	6.2	12.5
P. vulgaris (7)	HR 756	<0.01–12.5	0.05	0.4
	Carbenicillin	0.8–>400	25	25
	Cefoxitin	1.6–400	3.1	3.1
	Cefamandole	12.5–>400	400	>400
	Cefuroxime	50–>400	400	>400
P. rettgeri (11)	HR 756	<0.01–1.6	0.05	1.6
	Carbenicillin	0.02–>400	>400	>400
	Cefoxitin	1.6–400	12.5	100
	Cefamandole	0.2–100	25	100
	Cefuroxime	0.8–400	50	200
Providencia (15)	HR 756	<0.01–3.1	0.4	1.6
	Carbenicillin	0.8–>400	100	400
	Cefoxitin	1.6–200	6.2	12.5
	Cefamandole	0.8–>400	25	400
	Cefuroxime	1.6–100	25	100
Citrobacter (32)	HR 756	0.02–100	0.1	25
	Carbenicillin	0.4–>400	100	400
	Cephalothin	0.8–>400	25	400
	Cefamandole	0.2–>400	0.8	200
	Cefoxitin	0.8–>400	12.5	400
	Cefuroxime	0.8–>400	6.2	200
Salmonella (11)	HR 756	0.05–0.2	0.1	0.1
	Cephalothin	0.8–50	1.6	25
	Cefoxitin	1.6–6.2	1.6	3.1
	Cefamandole	0.2–25	0.8	12.5
	Cefuroxime	3.1–12.5	3.1	6.2
	Carbenicillin	3.1–>400	6.2	100
Shigella (16)	HR 756	<0.01–1.6	0.02	0.8
	Carbenicillin	0.2–>400	3.1	400
	Cephalothin	1.6–>400	6.2	400
	Cefoxitin	1.6–100	3.1	100
	Cefamandole	0.2–>400	0.8	25
	Cefuroxime	0.8–200	3.1	50

TABLE 1. *(continued)*

Organism (no. of strains)	Drug	MIC (μg/ml) range	MIC_{50} (μg/ml)	MIC_{90}
Serratia (32)	HR 756	0.2–200	50	100
	Cefoxitin	25–>400	400	>400
	Cefamandole	50–>400	400	>400
	Cefuroxime	100–>400	400	>400
	Carbenicillin	200–>400	400	400
Acinetobacter (17)	HR 756	0.05–200	25	200
	Carbenicillin	1.6–>400	50	400
	Cefoxitin	1.6–>400	200	400
	Cefamandole	3.1–400	100	400
	Cefuroxime	0.8–400	100	400
B. fragilis (30)	HR 756	3.1–200	25	100
	Carbenicillin	12.5–>400	50	200
	Cefoxitin	6.2–50	6.2	25
Bacteroides (21)	HR 756	0.2–100	50	100
	Cefoxitin	0.2–50	6.2	25
	Cephalothin	0.2–400	200	400
	Carbenicillin	25–400	100	400
	Penicillin G	3.1–>400	25	400
P. aeruginosa (123)	HR 756	0.4–>400	25	100
	Cefamandole	50–>400	400	>400
	Cefoxitin	50–>400	400	>400
	Carbenicillin	12.5–>400	25	400
	Piperacillin	0.4–400	6.2	25
	Gentamicin	0.4–400	1.6	400
	Amikacin	0.8–200	3.1	12.5
	Tobramycin	0.4–100	0.8	50
P. cepacia (7)	HR 756	6.2–100	100	100
	Cefoxitin	50–200	100	200
	Cefamandole	100–>400	200	>400
	Cefuroxime	25–>400	400	>400
	Carbenicillin	25–>400	25	>400
P. maltophilia (7)	HR 756	12.5–200	50	100
	Cefoxitin	200–>400	400	>400
	Cefamandole	400–>400	400	>400
	Cefuroxime	400–>400	>400	>400
	Carbenicillin	12.5–400	25	200

(2 isolates); and *Fusobacterium varium*, 200 μg/ml (1 isolate).

There was no major effect of growth medium upon MICs or MBCs of HR 756 in tests with MH broth, MH agar, nutrient broth, brain heart infusion broth, or Trypticase soy broth. Use of the low-ionic, low-osmolality medium such as nutrient broth did not reduce the MICs for either *Enterobacteriaceae* or *Pseudomonas*. The osmolality of nutrient broth was 60 mosM and conductivity was 1.5 mS, whereas the conductivity of MH broth was 440 mosM and conductivity was 12 mS. Similarly, differences in phosphate and calcium and magnesium content illustrated by the Trypticase soy and MH media did not affect the results. The effect of pH of the medium was determined by adjustment of the MH broth to pH 6, 7, and 8. *S. aureus*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa* showed identical or only twofold variation in both MICs and MBCs. Comparison of MICs obtained in MH agar with those obtained in MH broth revealed no differences for *E. coli*, *K. pneumoniae*, indole-positive *Proteus*, *Providencia*, *Serratia*, *Enterobacter*, *Pseudomonas*, and *S. aureus*.

The MBCs of HR 756 were identical or only twofold greater than the MICs for 60% of the isolates tested. Although the MICs of *E. coli*, *E. aerogenes*, *E. cloacae*, and *K. pneumoniae* were less than 1 μg/ml, the MBCs were not greater.

Similarly, two-thirds of the *P. aeruginosa* had MBCs equal to or only twofold greater than the MICs.

Inoculum size did have an effect on MICs for most of the species tested. Illustrative examples are given in Table 2. In general, there was a minor difference, twofold, between the HR 756 MIC at 10^3 and 10^5 CFU. However, at 10^7 CFU the MIC of HR 756 for many organisms was 8- to 128-fold greater. Nonetheless, in every species except *Serratia*, some of the 50 organisms tested had MICs which were identical at 10^3, 10^5, and 10^7 CFU. *Pseudomonas* showed a smaller increase in MICs as the number of CFU increased

TABLE 2. *Inoculum effect on MICs of HR 756*

Species	MIC (μg/ml) at:		
	10^7 CFU	10^5 CFU	10^3 CFU
E. coli	6.3	0.2	0.1
E. coli	0.05	0.05	0.05
K. pneumoniae	6.3	0.1	0.05
K. pneumoniae	0.01	0.01	0.01
P. vulgaris	25	12.5	6.3
P. rettgeri	200	12.5	0.8
P. morganii	25	12.5	6.3
Providencia	25	0.2	0.1
S. marcescens	>400	0.2	0.1
S. marcescens	>400	50	25
E. aerogenes	6.3	0.02	0.02
E. cloacae	25	0.2	0.1
E. hafnia	25	0.8	0.05
C. freundii	12.5	6.3	0.05
P. aeruginosa	25	6.3	6.3
P. aeruginosa	100	50	25
S. aureus	3.1	3.1	3.1
S. aureus	1.6	1.6	1.6

than did other species. Overall, 55% of the organisms tested showed MICs at 10^7 CFU which were 4-fold to 256-fold greater than the MICs at 10^5 CFU.

The MICs and MBCs of HR 756 in the presence of 50% human serum were compared with those in broth of *S. aureus*, *P. aeruginosa*, *Enterobacter*, and *E. coli*. MICs and MBCs in serum always were no greater than those found in broth alone.

The presence of β-lactamase activity did not affect MICs. The mean MIC for 10 *E. coli*, 10 *Klebsiella*, 10 *Citrobacter*, and 10 *Enterobacter* containing either plasmid or chromosomally mediated β-lactamases was 0.1 μg/ml, which was identical with the mean MIC of 40 matched strains which lacked β-lactamase detectable by use of a chromogenic substrate or the iodometric assay.

Table 3 shows the comparative activity of the newer cephalosporins against cephalothin-resistant isolates with MICs >200 μg/ml. The seven isolates with HR 756 MICs above 1 μg/ml had MICs greater than 25 μg/ml for the other new cephalosporins and carbenicillin.

Studies of the comparative activity of HR 756 and other β-lactam antibiotics were performed utilizing killing curves. Figure 1 shows that HR 756 was as effective as carbenicillin or piperacillin in killing *P. aeruginosa*. Indeed, with HR 756 there was the least regrowth of organisms. Comparison of the killing ability of cephalosporins and cephamycins against an *E. coli* with a plasmid-mediated β-lactamase is shown in Fig.

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

Species	HR 756	Cefoxitin	Cefamandole	Cefuroxime	Carbenicillin
P. stuartii	0.8	>200	>200	25	>400
Acinetobacter	0.1	3.1	12.5	12.5	>400
C. fruendii	0.2	3.1	12.5	25	200
E. coli	0.05	1.6	100	3.1	>400
K. pneumoniae	0.20	12.5	50	12.5	>400
E. aerogenes	0.025	>200	6.3	1.6	>400
E. cloacae	0.1	>200	>200	12.5	>400
P. mirabilis	3.1	25	100	50	>400
P. morganii	0.05	12.5	1.6	50	0.8
P. rettgeri	1.6	100	200	100	>400
P. vulgaris	0.4	3.1	200	200	>400
S. typhi	0.2	3.1	25	6.3	>400
S. sonnei	0.4	6.3	200	200	>400
S. marcescens	3.1	100	>200	>200	>400
S. marcescens	400	>400	>400	>400	>400
C. freundii	25	200	>200	100	>400
C. freundii	25	400	>200	100	>400
E. cloacae	50	>200	>200	>200	>200
P. vulgaris	12.5	>200	>200	>200	>200
S. marcescens	50	>400	>400	>400	>400

[a] Cephalothin MICs were >200 μg/ml.

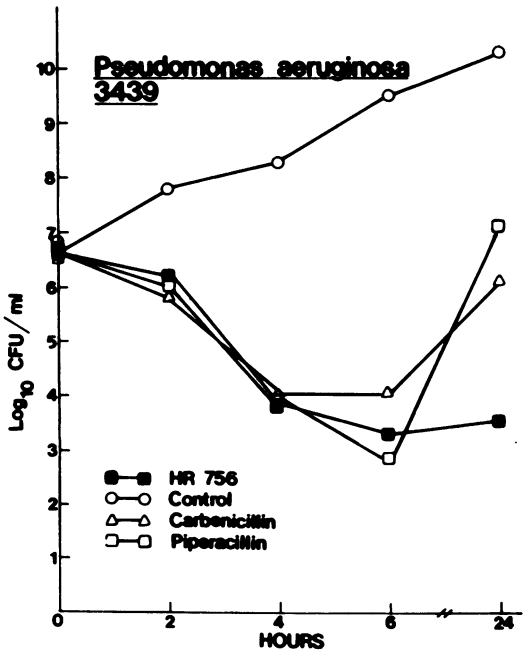

FIG. 1. *Comparative bactericidal activity of HR 756, carbenicillin, and piperacillin against P. aeruginosa. Each agent was present at twice the MIC: HR 756, 6.2 μg/ml; carbenicillin, 200 μg/ml; and piperacillin, 3.1 μg/ml.*

2. Each agent was present at a concentration twice the MIC. HR 756 at 0.2 μg/ml was as effective as the other agents at concentrations of 3.2 μg/ml for cefamandole to 25 μg/ml for cephalothin. Furthermore, with HR 756 the number of CFU was less at 24 h than at 6 h, which was not true for cephalothin or cefamandole. Although HR 756 had MICs against *S. aureus* which are 8- to 16-fold greater than those of cephalothin and cefamandole, it killed *S. aureus* at a rate comparable to the killing capacity of cephalothin and cefamandole (Fig. 3).

Synergistic activity. HR 756, when combined with aminoglycosides such as gentamicin, amikacin or netilmicin, showed a synergistic increase in activity against some *Enterobacteriaceae* and *P. aeurginosa*. The number of strains which showed synergy when HR 756 and gentamicin were combined is given in Table 4. Synergy was seen with *P. aeruginosa* and indole-positive *Proteus*, but most *E. coli* were so susceptible to HR 756 that synergy could not be demonstrated. No synergy was shown for the small number of *Serratia* tested. The combined activity of HR 756 and gentamicin would be best considered from the viewpoint that at 12.5 μg of HR 756 per ml 47% of *P. aeruginosa* were inhibited and at 3.1 μg of gentamicin per ml 62% were inhibited, but when combined 80% of the isolates were inhibited.

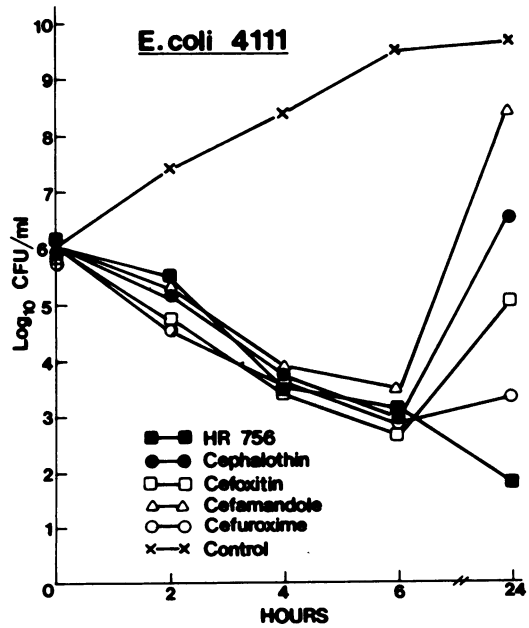

FIG. 2. *Comparative bactericidal activity of cephalosporins and cephamycins against E. coli. Each agent present at twice the MIC: HR 756, 0.1 μg/ml; cephalothin, 12.5 μg/ml; cefoxitin, 3.1 μg/ml; cefamandole, 0.1 μg/ml; and cefuroxime, 6.2 μg/ml.*

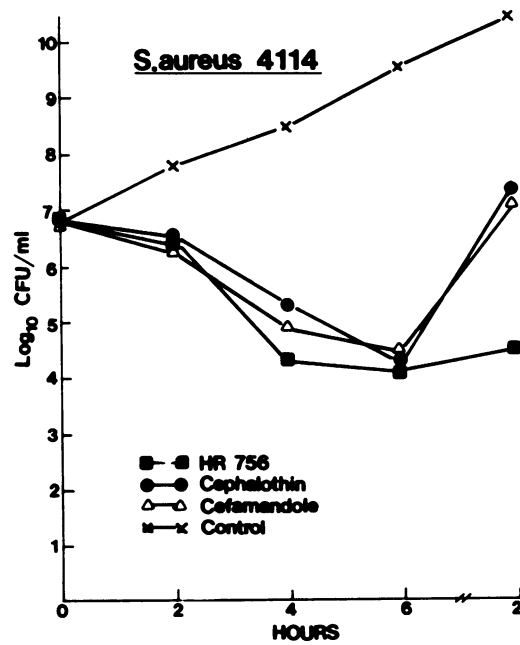

FIG. 3. *Comparative bactericidal activity of HR 756 against S. aureus. Concentrations were twice the MICs. MICs were as follows: HR 756, 3.1 μg/ml; cephalothin, 0.2 μg/ml; and cefamandole, 0.8 μg/ml.*

TABLE 4. *Synergistic activity of HR 756 combined with gentamicin*

Organism	No. of isolates	No. of isolates showing synergy
P. aeruginosa	60	34
E. coli	7	1
P. morganii	5	4
P. rettgeri	4	1
P. vulgaris	2	2
P. stuartii	5	0
K. pneumoniae	6	1
S. marcescens	7	0
S. aureus	6	1

Protein binding. The protein binding of HR 756 varied from 27% as determined by agar diffusion to 38% as determined by ultracentrifugation and dialysis.

DISCUSSION

Although major advances in cephalosporin antibiotic chemistry have occurred in the last few years, none of the major new agents—cefamandole (7), cefuroxime (12, 14), or cefoxitin (8)—has been active against *P. aeruginosa*. Each of these new agents is not hydrolyzed by many of the β-lactamases, whether plasmid or chromosomally mediated, which are present in most gram-negative species (1, 7, 8, 12). One cephalosporin, SCE 129, has been reported to inhibit *Pseudomonas* (16). But this agent, although resistant to hydrolysis by β-lactamases, has a markedly restricted spectrum of activity, because it does not inhibit most of the members of the *Enterobacteriaceae* (16; Neu and Fu, Antimicrob. Agents Chemother., in press).

This study demonstrates the impressive gram-positive and gram-negative spectrum of HR 756. HR 756 is the most potent β-lactam compound tested against members of the *Enterobacteriaceae*. It was as active as mecillinam against *E. coli* (10) and was more active than any of the new cephalosporins or penicillins against cephalothin-resistant *Klebsiella*, *Enterobacter*, and *Proteus* species (2–4). The activity is partially related to β-lactamase stability because, as we have already shown, the compound is not destroyed by gram-negative β-lactamases (5). However, HR 756 was as active against bacteria which lacked β-lactamases as it was against β-lactamase-containing isolates. Furthermore, the compound was active against organisms such as *E. cloacae* in which entry of a compound into the cell appears to be more important than β-lactamase stability, as evidenced by the difference in activity of cefoxitin and cefamandole against *Enterobacter* (7, 8).

The activity of HR 756 against *P. aeruginosa* was comparable to that of carbenicillin, mezlocillin, and ticarcillin (3, 4) but was not as active as piperacillin or azlocillin against *P. aeruginosa* (3, 4), nor was it as active as SCE-129, a unique cephalosporin active against *P. aeruginosa* and *S. aureus* (16). Unfortunately, the HR 756 MICs were high, >50 μg/ml, against most *P. aeruginosa* which had piperacillin MICs above 200 μg/ml. HR 756 had activity against gram-negative anaerobic organisms, which was slightly less than that of cefoxitin, but superior to other β-lactams.

HR 756 was much less active against gram-positive bacilli such as *Listeria* than it was against the gram-negative enteric organisms.

Overall, HR 756 is the most active cephalosporin tested to date. Preliminary studies of its human pharmacology by our group indicate that intravenous infusion will yield serum and urine levels comparable to those achieved with cefamandole and cefoxitin (11). The compound has been well tolerated in multiple doses in normal human volunteers and has produced no toxic side effects (Aswapokee, Fu, and Neu, manuscript in preparation).

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