Comparison of the In Vitro Activities of HR756 with Cephalothin, Cefoxitin, and Cefamandole

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The in vitro activity of HR756, a new semisynthetic cephalosporin, was compared with the activities of cephalothin, cefoxitin, and cefamandole against 1,535 isolates of gram-positive and gram-negative bacteria. HR756 was less active than cephalothin and cefamandole and twofold more active than cefoxitin against *Staphylococcus*. All four of the antibiotics were inactive against the enterococcus group of *Streptococcus*; however, HR756 was the most active antibiotic against the other isolates of *Streptococcus*. HR756 was also more active against isolates of *Enterobacteriaceae*, including 84 to 95% of the isolates resistant to one or more of the other three antibiotics. HR756, at a concentration of 12.5 µg/ml, inhibited 86, 75, and 100% of the isolates of *Pseudomonas aeruginosa*, other *Pseudomonas* species, and *Acinetobacter*, respectively. The minimal inhibitory concentrations and minimal bactericidal concentrations of HR756 were within one twofold dilution for 11 of 21 gram-positive cocci and 119 of 125 gram-negative bacilli tested.

Cephalosporins are broad-spectrum antibiotics with activity against most gram-positive and gram-negative bacteria. Unfortunately the emergence of multiply antibiotic-resistant bacteria, particularly among the gram-negative bacillus class, has become a serious therapeutic problem and has stimulated the search for newer antibiotics (2, 5). One such agent is HR756, a new semisynthetic cephalosporin which is resistant to all of the five classes of β-lactamases defined by Richmond (3; K. P. Fu and H. C. Neu, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, A10, p. 2). In this study the in vitro activity of HR756 was tested against 1,535 isolates of bacteria and compared with the activities of cephalothin, cefamandole, and the cephamycin antibiotic cefoxitin.

MATERIALS AND METHODS

Antibiotics. HR756 was obtained from Hoechst-Roussel Pharmaceutical, Inc., Sommerville, N. J.; cephalothin and cefamandole were obtained from Eli Lilly & Co., Indianapolis, Ind.; and cefoxitin was obtained from Merck, Sharp, & Dohme, West Point, Pa.

Organisms. The organisms used in this study were randomly selected recent isolates obtained from the Barnes Hospital Clinical Microbiology Laboratory, St. Louis, Mo.

Susceptibility tests. The minimal inhibitory concentrations (MICs) of the antibiotics were determined by broth microdilution. Serial twofold dilutions of the antibiotics were prepared in Mueller-Hinton broth

(Difco Laboratories, Detroit, Mich.), and 100 µl of each concentration was automatically delivered (MIC 2000 dispenser; Cooke Engineering Co. [Dynatech Corp.], Alexandria, Va.) into microtiter trays. The trays were stored at -20°C and used within 1 week of preparation. The inoculum was prepared by inoculating four or five colonies of the test organisms into tryptic soy broth and then incubating the cultures at 35°C for 2 to 4 h. Each suspension was adjusted to a turbidity equivalent of 0.5 of a McFarland no. 1 standard, and 1 µl of a 1:10 dilution of the adjusted suspension (10⁶ colony-forming units) was inoculated into each microtiter well. After overnight incubation at 35°C, the MICs were determined as the lowest concentration of antibiotic yielding no visibly detectable growth.

The minimal bactericidal concentrations (MBCs) of the antibiotics were determined by subculturing 0.01 ml of the broth from each well exhibiting no visible growth onto a culture plate of tryptic soy agar (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 5% sheep blood. After overnight incubation at 35°C, the MBCs were determined as the lowest concentration of antibiotic yielding no growth or only one colony, that is, the concentration at which at least 99.9% of the bacteria were killed.

RESULTS

The MICs of HR756, cephalothin, cefamandole, and cefoxitin which inhibited 50 and 90% of the 1,535 clinical isolates are summarized in Table 1. HR756 was more active than cefoxitin against *Staphylococcus aureus* and *Staphylo-*

coccus epidermidis but less active than cephalothin and cefamandole. At concentrations of 1.6 and 6.2 µg/ml, HR756 inhibited 90% of the *S. aureus* and *S. epidermidis* isolates, respectively. All four of the antibiotics were inactive against the enterococcus group of *Streptococcus*, with 25 µg/ml or greater required to inhibit 50% of the isolates. However, HR756 was the most active antibiotic against the other isolates of streptococci tested, with at least 90% inhibited by 0.8 µg of the antibiotic per ml.

HR756 was the most active antibiotic against isolates of the family *Enterobacteriaceae*, with 90% or more of the isolates of *Escherichia*, *Klebsiella*, *Enterobacter aerogenes*, *Citrobacter* spp., *Salmonella*, and *Proteus mirabilis* inhibited by the lowest concentration of HR756 tested (0.2 µg/ml). HR756 inhibited two of three isolates of *Providencia* at 0.2 µg/ml, all indole-positive *Proteus* isolates at 0.4 µg/ml, and 90% of the *Enterobacter cloacae* isolates at 1.6

µg/ml. At concentrations of 3.1 and 12.5 µg/ml, 81 and more than 90% of the *Serratia* isolates, respectively, were inhibited.

An interesting observation was the effectiveness of HR756 against *Pseudomonas aeruginosa*. At concentrations of 6.2 and 12.5 µg/ml, HR756 inhibited 50 and 86% of the isolates of *P. aeruginosa*, respectively. HR756 was also the most active antibiotic against other *Pseudomonas* species and *Acinetobacter*, of which 75 and 100% of the isolates, respectively, were inhibited at a concentration of 12.5 µg/ml.

The activity of HR756 against isolates of the *Enterobacteriaceae* which were resistant to cephalothin, cefoxitin, or cefamandole was also determined and compared with strains susceptible to these antibiotics (Table 2). For susceptible isolates (MICs ≤ 25 µg of cephalothin, cefoxitin, or cefamandole per ml), the minimal concentration of HR756 required to inhibit 90% of the isolates (MIC₉₀) was ≤0.2 µg/ml for all

TABLE 1. Comparative activity of HR756, cephalothin, cefoxitin, and cefamandole

Organism (no. tested)	HR756		Cephalothin		Cefoxitin		Cefamandole	
	MIC ₅₀ ^a (µg/ml)	MIC ₉₀ ^b (µg/ml)	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)
<i>Staphylococcus aureus</i> (172)	1.6	1.6	0.4	0.4	3.1	3.1	0.4	0.8
<i>Staphylococcus epidermidis</i> (107)	0.8	6.2	≤0.2	0.8	3.1	12.5	0.4	1.6
<i>Streptococcus</i> , enterococci (116)	>100	>100	25	50	>100	>100	25	50
<i>Streptococcus</i> , non-enterococci (23)	≤0.2	0.8	≤0.2	6.2	1.6	50	≤0.2	6.2
<i>Escherichia coli</i> (425)	≤0.2	≤0.2	3.1	12.5	1.6	6.2	0.4	3.1
<i>Klebsiella pneumoniae</i> (171)	≤0.2	≤0.2	1.6	25	1.6	6.2	0.8	12.5
<i>Enterobacter aerogenes</i> (51)	≤0.2	≤0.2	25	100	>100	>100	0.8	12.5
<i>Enterobacter cloacae</i> (49)	≤0.2	1.6	100	>100	>100	>100	3.1	>100
<i>Citrobacter diversus</i> (25)	≤0.2	≤0.2	1.6	3.1	1.6	3.1	0.4	1.6
<i>Citrobacter freundii</i> (18)	≤0.2	≤0.2	3.1	50	12.5	100	0.4	50
<i>Salmonella</i> (7)	≤0.2	≤0.2	12.5	12.5	3.1	3.1	6.2	6.2
<i>Proteus mirabilis</i> (129)	≤0.2	≤0.2	1.6	6.2	1.6	3.1	0.4	1.6
<i>Proteus</i> , indole positive (17)	≤0.2	0.4	>100	>100	3.1	12.5	6.2	100
<i>Providencia</i> (3)	≤0.2	12.5	>100	>100	3.1	>100	0.8	>100
<i>Serratia marcescens</i> (37)	≤0.2	12.5	>100	>100	12.5	100	25	>100
<i>Pseudomonas aeruginosa</i> (155)	6.2	25	>100	>100	>100	>100	>100	>100
<i>Pseudomonas</i> spp. (8)	12.5	50	>100	>100	>100	>100	>100	>100
<i>Acinetobacter</i> (22)	6.2	125	>100	>100	50	100	12.5	50

^a MIC₅₀, MIC required to inhibit 50% of the isolates tested.

^b MIC₉₀, MIC required to inhibit 90% of the isolates tested.

TABLE 2. *In vitro* activity of HR756 against isolates of Enterobacteriaceae susceptible and resistant to cephalothin, cefoxitin, or cefamandole

Organism	Antibiotic	Antibiotic-susceptible isolates ^a		Antibiotic-resistant isolates ^b	
		No.	HR756 MIC ₉₀ (μg/ml)	No.	HR756 MIC ₉₀ (μg/ml)
<i>Escherichia</i>	Cephalothin	376	≤0.2	49	1.6
	Cefoxitin	413	≤0.2	12	3.1
	Cefamandole	419	≤0.2	6	3.1
<i>Klebsiella</i>	Cephalothin	150	≤0.2	21	0.4
	Cefoxitin	164	≤0.2	7	1.6
	Cefamandole	155	≤0.2	16	0.4
<i>Enterobacter</i>	Cephalothin	29	≤0.2	71	25
	Cefoxitin	18	≤0.2	82	1.6
	Cefamandole	86	≤0.2	14	>100
<i>Citrobacter</i>	Cephalothin	38	≤0.2	4	25
	Cefoxitin	34	≤0.2	8	25
	Cefamandole	41	≤0.2	1	25
<i>Proteus</i>	Cephalothin	128	≤0.2	19	0.4
	Cefoxitin	143	≤0.2	3	≤0.2
	Cefamandole	142	≤0.2	2	0.4
<i>Serratia</i>	Cephalothin	6	25	31	6.2
	Cefoxitin	22	≤0.2	15	12.5
	Cefamandole	18	12.5	19	12.5

^a Isolates of *Enterobacteriaceae* which were inhibited by <25 μg of cephalothin, cefoxitin, or cefamandole per ml.

^b Isolates of *Enterobacteriaceae* which were inhibited by ≥25 μg of cephalothin, cefoxitin, or cefamandole per ml.

genera except *Serratia*. For resistant isolates (MICs ≥ 25 μg/ml), the MIC₉₀ of HR756 ranged from the lowest concentration tested (≤0.2 μg/ml) to the highest (≥100 μg/ml). Although HR756 was less active against the resistant isolates, the MIC₉₀ was ≤3.1 μg/ml for *Escherichia*, *Klebsiella*, and *Proteus* and ≤12.5 μg/ml for *Serratia*. The MIC of HR756 was ≤0.2 μg/ml for all but one isolate of *Citrobacter*, which had an MIC of 25 μg/ml. HR756 at a concentration of ≤0.2 μg/ml inhibited 77, 80, and 21% of the isolates of *Enterobacter* resistant to cephalothin, cefoxitin, and cefamandole. A total of 185 (95%) of the 195 isolates of *Enterobacteriaceae* which were resistant to cephalothin as defined by an MIC of ≥25 μg/ml were susceptible to HR756. A total of 118 (91%) of the 129 *Enterobacteriaceae* resistant to cefoxitin and 49 (84%) of the 58 isolates resistant to cefamandole were susceptible to HR756.

The MBC of HR756 was determined for 146 gram-positive cocci and gram-negative bacilli (Table 3). The MBC-MIC ratio for HR756 was 1 or 2 for 11 of the 21 gram-positive cocci and 119 of the 125 gram-negative bacilli. Greater than a one dilution difference (ratio greater than two) between MIC and MBC was noted for *S. aureus* (7 of 14 isolates), enterococci (all 3 isolates), *Pseudomonas* (3 of 20 isolates), *Enterobacter* (1 of 8 isolates), and *Proteus* (2 of 19 isolates).

TABLE 3. Comparison of MICs and MBCs of HR756 against gram-positive and gram-negative organisms

Organism	No. tested	No. with an MBC-MIC ratio of:				
		1	2	4	8	16 or more
<i>Staphylococcus aureus</i>	14	3	4	1	1	
<i>Staphylococcus epidermidis</i>	4	4				
<i>Streptococcus, enterococci</i>	3			1	1	1
<i>Pseudomonas</i>	20	8	9	1		2
<i>Escherichia</i>	37	36	1			
<i>Klebsiella</i>	21	21				
<i>Enterobacter</i>	8	7				1
<i>Citrobacter</i>	6	6				
<i>Proteus mirabilis</i>	15	13	1	1		
<i>Proteus, indole positive</i>	4	3		1		
Other gram-negative bacilli	14	10	4			

DISCUSSION

HR756 is a new semisynthetic cephalothin with activity against a broad range of gram-positive and gram-negative bacteria. Presumably the broad spectrum of activity is due to resistance to the five known β-lactamases (Fu

and Neu, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, A10, p. 2).

In the study reported herein HR756 was active against isolates of *Staphylococcus*, nonenterococcal *Streptococcus*, *Enterobacteriaceae*, and *Acinetobacter*. A most interesting finding was its apparent activity against *P. aeruginosa* and other *Pseudomonas* species.

In comparison with the other antibiotics which were tested, HR756 was less active than cephalothin and cefamandole against *Staphylococcus*; however, 97 to 99% of the isolates were inhibited by 6.2 μ g of HR756 per ml. HR756 was approximately twofold more active than cefoxitin against staphylococci. MBCs of HR756 for 6 of 14 isolates of *S. aureus* were more than fourfold greater than the MICs. This is similar to the proportion of clinical isolates of *S. aureus* which are tolerant to the bactericidal activity of cephalothin (1, 4).

HR756 was very active against isolates of *Enterobacteriaceae* which were both susceptible and resistant to the other antibiotics tested. This was particularly true with isolates of *Enterobacter*, *Citrobacter*, indole-positive *Proteus*, and *Serratia*, against which the other antibiotics were much less active. In addition, the MBC-MIC ratio of HR756 was 1 or 2 for all isolates of *Enterobacteriaceae* except one *Enterobacter* and two *Proteus*.

The activity of HR756 against *Pseudomonas*, particularly *P. aeruginosa*, and *Acinetobacter* was especially noteworthy, in that 77 to 100% of

the isolates were inhibited by ≤ 12.5 μ g/ml and the antibiotic was bactericidal for all but three isolates.

We have assumed that blood levels obtainable with HR756 are comparable to those achieved with the other cephalosporins. Preliminary data indicate that this is an accurate assumption (W. Novick, personal communication), but additional studies are needed to substantiate this. If the broad spectrum of activity is confirmed, this will be the first cephalosporin active against *Pseudomonas* and all genera of *Enterobacteriaceae*, as well as some gram-positive bacteria.

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