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HR 756, a Highly Active Cephalosporin: Comparison with Cefazolin and Carbenicillin

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HR 756, a new parenteral cephalosporin, was compared with cefazolin and carbenicillin for activity against a total of 264 strains of *Pseudomonas aeruginosa, Escherichia coli, Klebsiella* spp., *Proteus mirabilis, Proteus* spp. (indole positive), *Enterobacter* spp., *Salmonella typhi, Serratia marcescens, Providencia stuartii,* and *Staphylococcus aureus.* In every comparison, except that with the last organism, HR 756 was clearly more active than cefazolin and carbenicillin. All three compounds had similar activity against penicillin-susceptible staphylococci; against penicillin-resistant strains, HR 756 and cefazolin were equally active and superior to carbenicillin. HR 756 was compared with penicillin for activity against strains of *Streptococcus pyogenes,* Lancefield group D streptococci, and *Neisseria gonorrhoeae*; with ampicillin against *Haemophilus influenzae*; and with cefoxitin against *Bacteroides fragilis.* HR 756 was clearly more active than the respective reference compounds in all of these comparisons, except those involving the streptococci. HR 756 and penicillin were essentially equally active against *S. pyogenes*; against Lancefield group D, penicillin was 32 times as active as HR 756. HR 756 not only compared favorably with the reference compounds with respect to relative activity, but also effected growth inhibition of essentially all test organisms (*P. aeruginosa* and group D streptococci excepted) at remarkably low concentrations ranging from 0.015 to 2.0 μg/ml. A series of seven transfers of selected strains of *E. coli, Klebsiella* spp., *S. aureus,* and *P. aeruginosa* through medium containing HR 756 led to emergence of strains with significant levels of resistance to the agent. Resistance to HR 756 was retained for at least seven transfers through plain medium.

Although a number of new cephalosporins have been studied in recent years and antibodies such as cefoxitin (3) and cefuroxime (6), which are stable to a wide range of β-lactamases, have been introduced, there is a need for agents with an enlarged spectrum of activity. We were therefore interested in evaluating the in vitro activity of a novel cephalosporin for parenteral administration, HR 756, developed by Roussel Laboratories (for structure, see Fig. 1), against a wide range of pathogenic bacteria and comparing it with other β-lactam antibiotics. In particular, the activity against β-lactamase-producing strains was investigated. The development of in vitro resistance of a number of bacterial strains to HR 756 was also studied.

MATERIALS AND METHODS

Organisms. The in vitro susceptibilities of a wide range of recent bacterial isolates from Dudley Road Hospital were evaluated. All strains were identified by the API (API Laboratory Products Ltd., United Kingdom) method. Two strains of *Pseudomonas aeruginosa* known to be Tem$^+$ were obtained from E. Lowbury (Birmingham, England). A β-lactamase-positive strain of *Neisseria gonorrhoeae* was obtained from A. Percival (Liverpool, England). The production of β-lactamase by certain strains was verified by the chromogenic cephalosporin substrate nitrocefin (4).

Antimicrobial agents. These were obtained as follows: HR 756 (potency, 1,000 μg/mg) from Roussel Laboratories, Wembley, Middlesex, United Kingdom; benzylpenicillin (potency, 995 μg/mg), ampicillin (potency, 855 μg/mg), and carbenicillin (potency, 810 μg/mg) from Beecham Research Laboratories, Brentford, Middlesex, United Kingdom; cefazolin (potency, 928 μg/mg) from Eli Lilly & Co., Basingstoke, United Kingdom; and cefoxitin (potency, 950 μg/mg) from Merck Sharp & Dohme, Hoddesdon, United Kingdom.

Methods. Sensitivity testing was performed by a routine agar plate dilution procedure using Isosensitest agar, pH 7.2 (Oxoid, Basingstoke, United Kingdom). The *Enterobacteriaceae, P. aeruginosa, Staphylococcus aureus,* streptococci, and *Streptococcus pneumoniae* were tested against HR 756, carbenicillin, and cefazolin. The Isosensitest agar was supplemented as follows: with 5% lysed human blood (to support the growth of *Bacteroides fragilis*), a Levinthal preparation (to support the growth of *Haemophilus influenzae*), and a chocolate agar preparation (to support the growth of *N. gonorrhoeae*).

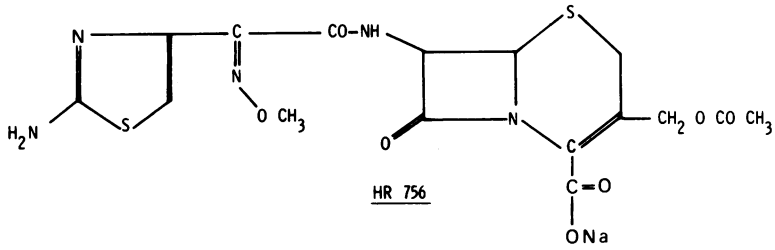

FIG. 1. *Structure of HR 756, 7-[2-(2-amino-4-thiazolyl)-2-(Z)-methoximino)-acetamido]-cephalosporanic acid.*

Inocula were prepared as follows. For all strains except those of *B. fragilis,* streptococci, *N. gonorrhoeae,* and *H. influenzae,* the organisms were grown overnight in nutrient broth yielding a viable count of about 10^9 colony-forming units (CFU) per ml. Strains of *B. fragilis* were grown overnight in thioglycolate broth, streptococci were grown in Todd-Hewitt broth, and *H. influenzae* was grown in Levinthals broth yielding about the same viable count. Strains of *N. gonorrhoeae* were grown overnight on a chocolate agar plate, and the growth was removed and resuspended in peptone–water just before use so as to yield a viable count of 10^6 to 10^7 CFU/ml. Two inocula (10^3 and 10^6 CFU) were employed in the susceptibility tests of all organisms except *N. gonorrhoeae.* These were obtained by transferring 1 μl of an undiluted and a 1:1,000 dilution of the culture to the antibiotic-containing media by a Denley (Denley-Tech Ltd., Billingshurst, United Kingdom) multipoint inoculating device. Strains of *N. gonorrhoeae* were tested undiluted and at a dilution of 1:10, so as to yield a final inoculum of 10^3 to 10^4 and 10^2 to 10^3 CFU.

All plates were incubated for 24 h at 37°C. The anaerobes were incubated in a GasPak (Baltimore Biological Laboratory, Cockeysville, Md.) jar and *H. influenzae* and *N. gonorrhoeae* in a 10% CO_2 incubator. The minimum inhibitory concentrattion (MIC) was defined as the micrograms of antimicrobial per milliliter of medium at which there was an estimated 99% reduction in the original inoculum.

Strains of *P. aeruginosa, Klebsiella* spp., *Escherichia coli* and *S. aureus,* selected on the basis of spectrum of susceptibility to HR 756, were passaged seven times on media containing varying amounts of HR 756 by using a gradient plate technique. After passage the MICs of HR 756 were determined by the above method. After seven additional passages on antibiotic-free media, the MICs were repeated.

RESULTS

Table 1 summarizes the MICs obtained for the 349 strains tested at the lower inoculum of 10^3 CFU. HR 756 has a high degree of activity against the *Enterobacteriaceae* tested, being more than 64-fold more active than cefazolin or carbenicillin. The majority of strains were susceptible to 0.06 μg or less of HR 756 per ml. This high activity was also apparent against *Serratia marcescens* and *Provedencia stuartii* which were resistant to cefazolin and carbenicillin. The high degree of activity against *Salmonella typhi* is also noteworthy.

HR 756 was 4 to 16 times more active than carbenicillin against *P. aeruginosa,* although these strains were less susceptible than the *Enterobacteriaceae.* Two Tem^+ strains of *P. aeruginosa* highly resistant to carbenicillin (MIC > 1,024 μg/ml) were inhibited by 4 and 8 μg of HR 756 per ml.

HR 756 was also highly active against *H. influenzae,* the strains being 16- to 32-fold more susceptible to this agent than to the reference compound ampicillin. Two β-lactamase-producing strains were included and were as susceptible to HR 756 as were the non-beta-lactamase producers.

The high activity of HR 756 also extended to *N. gonorrhoeae,* which was uniformly susceptible to 0.002 μg of HR 756 per ml. A β-lactamase-producing strain was inhibited by 0.008 μg of HR 756 per ml and 1 μg of benzylpenicillin per ml. Among the gram-positive organisms tested, Lancefield group A streptococci and *S. pneumoniae* were highly susceptible to both HR 756 and benzylpenicillin. In contrast fecal streptococci (Lancefield group D) were markedly less susceptible to HR 756 than to benzylpenicillin.

Cefazolin was marginally the most active compound when tested against *S. aureus.* There was little difference between the relative activity of cefazolin and HR 756 against both the penicillin-susceptible and -resistant strains. Carbenicillin showed decreased activity against the penicillin-resistant *S. aureus.*

Cefoxitin was more active than HR 756 against *B. fragilis,* the only anaerobe tested.

The effect of a 1,000-fold rise in inocula (from 10^3 to 10^6 CFU) is shown in Table 2. It would appear that the activity of HR 756 is only minimally reduced at the higher as compared with the lower inoculum against the majority of strains tested. The only exception was *B. fragilis,* where the activity of the reference compound cefoxitin was not affected by an inoculum increase, but there was an eightfold decrease in

TABLE 1. *MICs required to inhibit cumulative percentage of isolates (inoculum, 10^3 CFU)*

Organism (no. of isolates)	Antibiotic	MIC (μg/ml) for:		
		50% inhibition	75% inhibition	90% inhibition
P. aeruginosa (74)	HR 756	8	16	64
	Cefazolin	>256	>256	>256
	Carbenicillin	32	128	256
E. coli (40)	HR 756	<0.06	<0.06	0.12
	Cefazolin	1	4	64
	Carbenicillin	8	>256	>256
Klebsiella spp. (40)	HR 756	<0.06	<0.06	0.12
	Cefazolin	2	4	8
	Carbenicillin	>256	>256	>256
P. mirabilis (24)	HR 756	<0.06	<0.06	0.12
	Cefazolin	4	4	16
	Carbenicillin	0.5	>256	>256
Indole-positive *Proteus* spp. (25)	HR 756	<0.06	<0.06	0.12
	Cefazolin	64	128	>256
	Carbenicillin	0.5	2	>256
Enterobacter spp. (11)	HR 756	<0.06	<0.06	0.12
	Cefazolin	8	16	32
	Carbenicillin	2	2	>256
S. typhi (5)	HR 756	<0.06	<0.06	<0.06
	Cefazolin	1	1	1
	Carbenicillin	1	1	2
S. marcescens (8)	HR 756	<0.06	0.12	0.12
	Cefazolin	>256	>256	>256
	Carbenicillin	2	2	4
P. stuartii (25)	HR 756	0.12	0.25	0.5
	Cefazolin	8	16	32
	Carbenicillin	>256	>256	>256
S. aureus, penicillin susceptible (7)	HR 756	1	1	2
	Cefazolin	0.25	0.25	0.5
	Carbenicillin	0.5	0.5	1
S. aureus, penicillin resistant (5)	HR 756	1	2	2
	Cefazolin	0.25	0.25	1
	Carbenicillin	2	2	8
S. pyogenes (5)	HR 756	0.008	0.015	0.015
	Penicillin	0.004	0.008	0.008
Fecal streptococci, Lancefield group D (10)	HR 756	2	32	64
	Penicillin	1	2	2
H. influenzae (25)	HR 756	0.004	0.008	0.015
	Ampicillin	0.12	0.12	0.12
N. gonorrhoeae (12)	HR 756	0.002	0.002	0.002
	Penicillin	0.03	0.03	1
B. fragilis (33)	HR 756	2	2	2
	Cefoxitin	4	4	8

TABLE 2. *Mean increase in MIC associated with a 1,000-fold increase in inoculum*

Organism (no.)	Mean increase in MIC of:					
	HR 756	Cefazolin	Carbenicillin	Ampicillin	Cefoxitin	Penicillin
E. coli						
β−[a] (20)	2	2	2			
β+ (20)	2	8	32			
P. mirabilis						
β− (16)	2	2	2			
β+ (8)	2	8	32			
Klebsiella spp. (40)	2	8	NT[b]			
S. aureus						
β− (7)	2	2	2			
β+ (5)	2	2	64			
P. aeruginosa (74)	2	NT	2			
H. influenzae						
β− (23)	2			2		
β+ (2)	2			16		
B. fragilis (33)	8		NT		2	
N. gonorrhoeae						
β+ (1)	0		NT			64

[a] β+, Beta-lactamase-producing strains; β−, non-beta-lactamase-producing strains.
[b] NT, Not tested.

the activity of HR 756. The two β-lactamase-producing strains of *H. influenzae* tested showed a marked inoculum effect with ampicillin, but this was not seen with HR 756.

Table 3 shows that after seven transfers on media containing increasing amounts of HR 756, high-level resistance occurred in six of the eight strains of *P. aeruginosa* tested. A significant decrease (greater than fourfold) in susceptibility was noted in both strains of *E. coli*, one strain of *S. aureus* (no. F 105), and two strains of *Klebsiella* spp. (H4 and H34). The subsequent seven transfers on antibiotic-free medium did not indicate any significant change in susceptibility to HR 756 of the resistant strains.

TABLE 3. *Effect of seven passages on HR 756-containing media on the susceptibility of bacterial strains to HR 756*

Organism	MIC (μg/ml) of HR 756		
	Pre-transfer	Post-transfer	After 7 transfers on antibiotic-free medium
E. coli I 1	0.06	2	2
E. coli I 28	0.12	2	NT
Klebsiella H 4	0.06	2	4
Klebsiella H 34	1.0	16	NT
Klebsiella H 12	0.06	0.25	NT
S. aureus F 105	8	>256	256
S. aureus F 95	2	2	4
S. aureus F 168	1	4	NT
P. aeruginosa G 83	8	256	512
P. aeruginosa G 155	16	>1,024	512
P. aeruginosa G 20	16	>1,024	512
P. aeruginosa G 14	64	>1,024	512
P. aeruginosa G 8	16	8	32
P. aeruginosa G 24	16	8	32
P. aeruginosa G 11	16	256	NT
P. aeruginosa G 4	16	>1,024	NT

DISCUSSION

The broad antibacterial spectrum of the cephalosporins has contributed to their high clinical use. Unlike the available cephalosporins, HR 756 was consistently and considerably more active against a wide range of pathogenic bacteria than cefazolin, the reference compound in this study. HR 756 was also more active than the recent cephalosporins, cefuroxime (2, 5), cefoxitin (3), and cefamandole (2). In addition HR 756 has modest activity against *P. aeruginosa*, comparable to the α-amino-substituted penicillin mezlocillin (1, 9). Other pathogens, often considered as "problem" organisms in hospital practice (such as *S. marcescens, P. stuartii,* and *Klebsiella* spp.) were also extremely susceptible to HR 756. It was also of interest to note that HR 756 was extremely active against *S. typhi,* being

more active than mecillinam (8). The two common respiratory tract pathogens *S. pneumoniae* and *H. influenzae* were also extremely susceptible to HR 756, including the important β-lactamase-producing strains of the latter. HR 756 exhibited poor activity against fecal streptococci and a degree of activity similar to cefazolin against *S. aureus*.

These in vitro data would suggest that a use could be found for HR 756 in the treatment of a wide variety of serious infections, possibly as the sole agent instead of the usual regimes at present which combine an aminoglycoside with another antimicrobial.

The activity of any β-lactam antibiotic is only one facet of its in vitro properties. Stability to β-lactamase hydrolysis has considerable clinical importance. The evidence from this study suggests that HR 756 could be hydrolyzed by certain β-lactamases, although it would appear that the most prevalent type III or Tem enzyme does not significantly hydrolyze HR 756. This is supported by the observation that the Tem^+ strains of *P. aeruginosa, N. gonorrhoeae,* and *H. influenzae* remain highly susceptible even at the higher inoculum. In the case of *B. fragilis,* it is important to note that the β-lactamases present in these organisms (7) may hydrolyze HR 756 but not cefoxitin.

Passage of a variety of strains on media containing HR 756 indicated that resistance could readily be induced in vitro and that this resistance was stable on serial transfer on antibiotic-free media. In our experience this can be observed with carbenicillin, and the clinical relevance is doubtful.

Preliminary data (from Roussel Laboratories, Wembley, United Kingdom) suggest that this compound has a similar pharmacology to the currently available parenteral cephalosporins, the serum half-life being about 100 min and some metabolism with acetylation occurring. This implies that as the majority of common organisms (with the exception of *P. aeruginosa, S. aureus,* and group D streptococci) are susceptible to remarkably low concentrations of HR 756, it should be possible to use considerably lower doses of this compound, compared with cephalosporins in current use, when treating susceptible infections. It is possible that the dosing interval could be increased from the 6 or 8 h now usually used for a cephalosporin like cefazolin. The implications of using such an active agent in renal failure are also raised and require careful pharmacological evaluation.

HR 756, therefore, represents a significant advance in antimicrobial therapy, and the results of pharmacological and clinical studies are awaited with great interest.

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