IN VITRO ACTIVITY OF HR 756, A NEW CEPHALOSPORIN COMPOUND*

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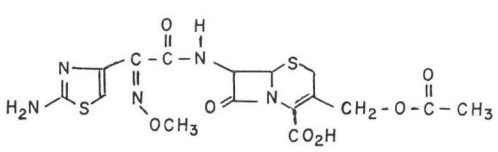
(Received for publication July 18, 1978)

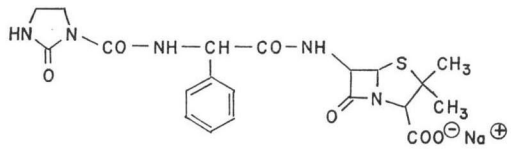
The *in vitro* activity of HR 756, a new cephalosporin, has been determined against recent clinical isolates and compared with that of other β-lactam antibiotics. The geometric means of the minimum inhibitory concentrations (MIC) for different isolates of *Escherichia coli* (100 isolates), *Klebsiella pneumoniae* (84), *Pseudomonas aeruginosa* (121), *Proteus mirabilis* (52), indole-positive *Proteus* species (9), *Salmonella* species (19), *Staphylococcus aureus* penicillin-sensitive (29) and penicillin-resistant (39) were: 0.095, 0.124, 11.1, 0.095, 0.0107, 0.078, 1 and 0.95 mcg/ml, respectively. Its activity was affected by rise in inoculum against *S. aureus* and *P. aeruginosa* but not against *K. pneumoniae* and *E. coli*. Bactericidal activity was determined by membrane filtration method. HR 756 was found to be bactericidal to *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *Proteus* species. Although the MICs of the tested *S. aureus* strains were 1 mcg/ml, 5 mcg/ml of HR 756 failed to kill 99% of the inoculum within 24 hours.

HR 756 (Fig. 1), the sodium salt of 7-[(2-(2-amino-4-thiazolyl)-2(Z)-methoximino)-acetamido]-cephalosporanic acid, is a cephalosporin compound which is highly active against both Gram-negative rods and Gram-positive cocci.[1,3,4] Its activity was compared with that of cephalothin, cefazolin, cefuroxime and cefoxitin, and carbenicillin, and azlocillin, a new ureido-penicillin[2].

Fig. 1.



HR 756



Azlocillin

Materials and Methods

All strains were of human origin, isolated and identified in our laboratory.

HR 756 was supplied by Hoechst AG, cephalothin by E. Lilly, cefazolin by Boehringer Mannheim, cefuroxime by Glaxo/Hoechst AG and cefoxitin by Merck, Sharp & Dohme. Azlocillin and carbenicillin were obtained from Bayer AG. Stock antibiotic solutions were prepared each day.

Minimum inhibitory concentrations (MIC) were determined by an agar dilution technique using D.S.T. agar (Oxoid), except for *Proteus* species, where KLED agar (Oxoid) was used.[10] The inoculum size was approximately 10^5 colony forming units/ml (CFU/ml). A multi-point inoculator was used to inoculate the plates.

Effect of inoculum: The effect of inoculum size on the MIC of HR 756 was determined using three different inocula: 10^3, 10^5 and 10^7 CFU/ml. The tables show the geometrical means of the MIC's for different isolates.

* This paper was presented in parts at the American Society of Microbiology Annual Meeting, Las Vegas, 1978

Bactericidal activity: Bactericidal activity was evaluated by the membrane filtration technique, described by KLEIN[5], OBERZILL[6] and VÖMEL[9] but was modified in our laboratory.[7,8]

A half ml of a 10^2 diluted four-hour culture of the test strain was added to a flask containing 49.5 ml of the Antibiotic Medium 3 (Difco) and the appropriate concentration of the antibiotic. Colony counts were performed at 0, 30 minutes, 1, 2, 4, 8 and 24 hours. The filters (Sartorius filter, grade 0.2 μm, 50 mm diameter) were cultured on Standard I Agar (Merck) or KLED agar (for *Proteus* species) at 37°C for 18~24 hours. Colony forming units surviving after exposure to HR 756 were defined in percentage of inoculum and plotted against time.

Results

The results of the *in vitro* susceptibility testing of isolates to HR 756 are summarized in Tables 1~3. HR 756 was more active than cephalothin, cefazolin, cefuroxime and cefoxitin against all Gram-negative

Table 1. Comparison of HR 756, cephalothin, cefazolin, cefuroxime and cefoxitin against clinical isolates.

Organism	% strains inhibited	Concentration (mcg/ml) required to inhibit given % of strains				
		HR 756	Cephalothin	Cefazolin	Cefuroxim	Cefoxitin
Staphylococcus aureus (29)* (penicillin-sensitive)	50	1	0.5	0.125	0.5	2
	90	2	4	1	4	4
Staphylococcus aureus (39) (penicillin-resistant)	50	1	0.5	0.25	0.5	2
	90	2	2	1	4	32
Escherichia coli (100)	50	<0.125	8	1	4	4
	90	0.5	32	4	8	16
Klebsiella pneumoniae (84)	50	<0.125	64	16	4	8
	90	0.5	>128	>128	32	128
Proteus mirabilis (52)	50	<0.125	1	0.5	0.5	0.5
	90	0.25	32	8	4	32
Salmonella species (19)	50	<0.125	2	0.5	4	1
	90	0.125	8	1	4	8

* Number of clinical isolates tested.

Table 2. Comparison of HR 756, carbenicillin and azlocillin against 121 strains of *Pseudomonas aeruginosa*.

Organism	% strains inhibited	Concentration (mcg/ml) required to inhibit given % of strains		
		HR 756	Carbenicillin	Azlocillin
Pseudomonas aeruginosa	50	8	32	4
	90	32	256	16

Table 3. Minimum inhibitory concentration against indole-positive *Proteus* species.

Strain number	Species	HR 756	Cefazolin	Cefuroxime	Cefoxitin	Cephalothin
387	*P. vulgaris*	0.03	128	16	4	>128
9223	*P. vulgaris*	<0.015	64	64	1	32
10	*P. vulgaris*	2	64	64	128	64
4887	*P. vulgaris*	<0.015	16	16	1	16
9219	*P. rettgeri*	<0.015	128	32	4	>128
58	*P. rettgeri*	<0.015	32	16	2	128
5482	*P. rettgeri*	<0.015	32	8	1	32
2634	*P. morganii*	<0.015	128	8	8	>128
5889	*P. morganii*	0.03	64	128	8	>128

Table 4. Time (T99) needed by 2 mcg/ml HR 756 to kill 99% of *E. coli*.

Strain	MIC	Inoculum	T99 in minutes
8156	0.06	2.4×10^5	50
913	0.06	1.8×10^5	45
1176	0.06	2.2×10^5	80
4112	0.06	3.9×10^5	90
4036	0.06	5.1×10^5	110

Table 5. Time (T99) needed by 3 mcg/ml HR 756 to kill 99% of *K. pneumoniae*.

Strain	MIC	Inoculum	T99 in minutes
1	0.03	5.9×10^5	95
3	0.03	3.9×10^5	105
5	0.03	5.3×10^5	180
25	0.03	2.8×10^5	95
21	0.03	3.9×10^5	135

Table 6. Time (T99) needed by 1 mcg/ml HR 756 to kill 99% of *Proteus* species.

Strain	Indole	MIC	Inoculum	T99 in minutes
3913	−	0.01	3.6×10^6	105
4323	−	0.01	1.0×10^6	75
4887	+	0.01	2.3×10^6	105
9223	+	0.01	1.4×10^6	120

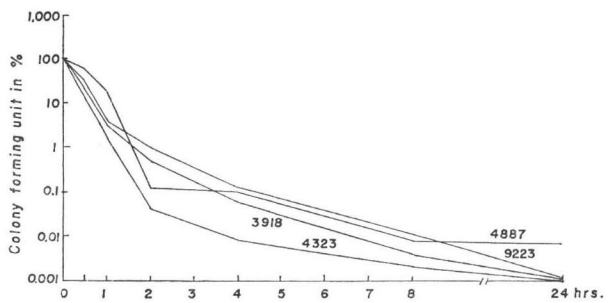
Table 7. Time (T99) needed by 30 mcg/ml HR 756 to kill 99% of *Pseudomonas aeruginosa*.

Strain	MIC	Inoculum	T99 in minutes
3963	8	5.6×10^5	80
6723	8	5.3×10^5	—
4614	8	4.0×10^6	165
5946	8	4.5×10^5	465
1106	8	3.8×10^6	165

Table 8. Geometric mean of minimum inhibitory concentration of HR 756 at 3 different inocula. (CFU/ml=colony forming units per milliliter) (n=number of isolates tested)

Organism		10^3	10^5	10^7
Escherichia coli	(n=9)	0.0675	0.0675	0.079
Klebsiella pneumoniae	(n=10)	0.102	0.088	0.117
Pseudomonas aeruginosa	(n=11)	6.22	12.44	19.33
Staphylococcus aureus	(n=10)	0.088	0.33	1.41

Fig. 2. Bactericidal activity of 1 mcg/ml HR 756 against *Proteus* species (indole-positive 4887, 9223; and indole-negative 3918, 4323). Viable counts *via* membrane filtration in Antibiotic Medium 3.



isolates listed in Tables 1 and 3. Its activity against penicillin-sensitive and penicillin-resistant strains of *S. aureus* was less than that of cephalothin and cefazolin. Against *P. aeruginosa* azlocillin was more active than HR 756, which was more active than carbenicillin on a weight basis.

Fig. 3. Bactericidal activity of 30 mcg/ml HR 756 against *Pseudomonas aeruginosa* (n=5). Viable counts *via* membrane filtration in Antibiotic Medium 3.

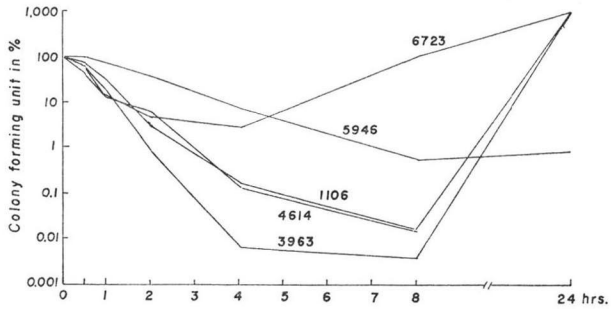


Fig. 4. Bactericidal activity of 5 mcg/ml HR 756 against *Staphylococcus aureus*. Viable counts *via* membrane filtration in Antibiotic Medium 3. Minimum inhibitory concentrations for all strains 1 mcg/ml.

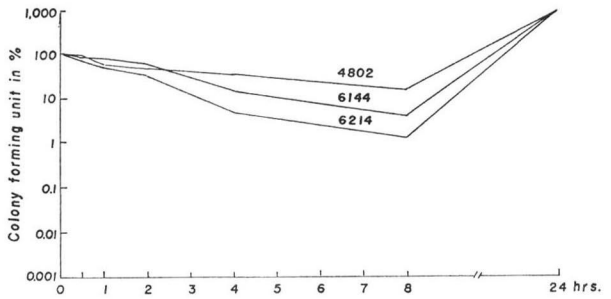


Table 9. Geometrical mean of the minimum inhibitory concentration in mcg/ml (n. d.=not determined). (n=number of isolates tested)

Organism	n	HR 756	Cephalothin	Cefazolin	Cefuroxime	Cefoxitin	Ampicillin	Azlocillin	Carbenicillin
S. aureus (penicillin sensitive)	29	1	1.10	0.28	1.13	2.60	n.d.	n.d.	n.d.
S. aureus (penicillin resistant)	39	0.95	0.79	0.25	0.93	3.23	n.d.	n.d.	n.d.
Salmonella species	19	0.078	2.31	0.72	3.72	2.31	4.15	n.d.	n.d.
Proteus mirabilis	52	0.095	2.28	1.45	0.74	1.55	n.d.	n.d.	n.d.
Proteus indole+	9	0.0107	80.6	43.5	25.4	4.0	n.d.	n.d.	n.d.
K. pneumoniae	84	0.124	32.0	14.73	6.73	10.42	n.d.	n.d.	n.d.
E. coli	100	0.095	9.38	1.61	3.97	3.89	n.d.	n.d.	n.d.
P. aeruginosa	121	11.1	n.d.	n.d.	n.d.	n.d.	n.d.	4.9	63.6

Fig. 2 shows the bactericidal activity of HR 756 against *Proteus* species. Similar curves were also seen for *K. pneumoniae* and *E. coli*. Tables 4 ~7 give the time needed to kill 99% of the inoculum. Against *P. aeruginosa* HR 756 needed more time to kill 99% of the inoculum (Table 7). In fact against one strain HR 756 failed to kill 99% of the inoculum. Fig. 4 shows the bactericidal effect of HR 756 at different times against *S. aureus*. It is obvious that the bactericidal activity against *S. aureus* strains is poor.

Table 8 lists the geometric means of MICs at different inocula. Only with *S. aureus* and more markedly with *P. aeruginosa* was there a rise in MIC with an increase in the size of the inoculum.

Discussion

Of the cephalosporins, including the cephamycin and cefoxitin compared in this study, HR 756 was the most active compound against enterobacteriaceae. Table 9 gives the geometrical means of the MICs determined. Against *S. aureus* strains (both penicillin-sensitive and resistant) the geometrical mean of the MICs was <1 mcg/ml. Cephalothin and cefazolin were more active than HR 756, especially against penicillin-resistant *S. aureus* strains. Here, cefoxitin was the least active compound. It is noteworthy that although the MIC against *S. aureus* was low, HR 756 failed to bring about any marked reduction of the inoculum (Fig. 4).

The most striking result was that HR 756 was more active against *P. aeruginosa* than carbenicillin on a weight basis, azlocillin being more active. HEYMÈS, LUTZ and SCHRINNER have reported similar results[3].

The activity of HR756 was affected by inoculum size only against *S. aureus* and *P. aeruginosa*, whereas increasing the inoculum from 10^3 to 10^7 CFU/ml of *E. coli* and *K. pneumoniae* did not change the MIC significantly.

The first *in vitro* experience against clinical isolates with this new compound is promising and our first clinical trials show HR 756 to be effective and safe. Further studies are recommended.

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