HRE 664, A NEW PARENTERAL PENEM

I. ANTIBACTERIAL ACTIVITY *IN VITRO*

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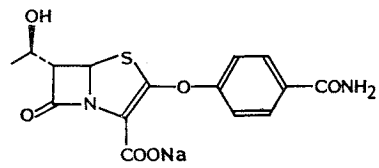
The new penem antibiotic HRE 664 displays potent antibacterial activity *in vitro* against a broad spectrum of clinically relevant bacterial strains including Gram-negative and Gram-positive aerobes and anaerobes.

With an MIC 90% of 0.43 μg/ml, it is also active against methicillin-resistant staphylococci. HRE 664 is extremely stable against β-lactamases, it binds preferentially to the penicillin-binding proteins 2, 3, 5 and 6 of *Escherichia coli*.

Besides penicillins and cephalosporins there are other antibacterial compounds such as the penems, which also possess the β-lactam-ring. In recent years there has been great interest in exploiting the modification of these compounds. Although many have been synthesized, only a few were submitted to broad preclinical and clinical investigations. Penem antibiotics are characterized by broad antibacterial spectra, including both aerobic and anaerobic Gram-positive and Gram-negative species[1~3].

Fig. 1.



HRE 664

Here we report on the *in vitro* antibacterial activity of the new parenteral penem HRE 664 (sodium 5(*R*),6(*S*)-3-(4-carbamoylphenoxy)-6-(1(*R*)-hydroxyethyl)-7-oxo-4-thia-1-azabicyclo[3,2,0]-hept-2-ene-2-carboxylate, Fig. 1).

Materials and Methods

Test Strains

The bacterial strains used were clinical isolates collected between 1978 and 1983 from various hospitals in Europe together with laboratory strains maintained in our department. All bacterial strains were stored on agar slants at room temperature or in liquid nitrogen.

Antibiotics

HRE 664 was synthesized by Hoechst UK, Milton Keynes. All other antibiotics used are commercially available.

Susceptibility Testing

The susceptibility of aerobic and anaerobic bacteria was tested using an agar dilution method with Mueller-Hinton agar and Wilkins-Chalgren agar, respectively[4].

Influence of Inoculum Size

The influence of the inoculum size on the activity of HRE 664 was examined in serial dilution tests in Mueller-Hinton medium (Difco). Five simultaneously prepared geometrical dilution series of the compound were inoculated with suspensions containing different densities of the test organism. The initial counts were approximately 1×10^2, 10^3, 10^4, 10^5 and 10^6 cfu/ml of nutrient medium.

Influence of the pH of the Culture Medium

The effect of the pH on the activity of HRE 664 wasdeter mined in heart infusion medium (Difco) which had been adjusted to values between pH 5.5 and pH 9. The MICs of HRE 664 for the test strains were determined in this medium by the serial dilution method.

Killing Curves

Mueller-Hinton medium was inoculated with the individual test strains and incubated at 37°C on a rotary shaker. After 2 hours HRE 664 was added to the cultures in different concentrations. Samples were withdrawn at intervals, diluted with saline and spread on agar plates. After 18 hours at 37°C the number of cfu/ml were counted.

Induction of Resistance *In Vitro*

A dilution series of HRE 664 in Mueller-Hinton medium was inoculated with one of the test strains (*Staphylococcus aureus* SG 511, *Salmonella typhimurium*) and incubated at 37°C. After 18 hours, bacteria from the test tube containing HRE 664 at a sub-inhibitory concentration and showing a dense growth were used to inoculate a fresh dilution series. The two test strains were passaged 30 times in this manner.

β-Lactamase Stability

The enzymes used were released from the bacteria by ultra-sonication and partially purified by fast protein liquid chromatography (FPLC)-chromatography on Superose (Pharmacia). HRE 664 was incubated with β-lactamase and at given time intervals samples were checked for their antibiotic content by the agar diffusion test.

Affinity for Penicillin-binding Proteins (PBPs)

The binding of HRE 664 to PBPs of *Escherichia coli* K-12 was determined using [^{125}I]ampicillin[5].

Results

Activity against Aerobic and Facultative Anaerobic Bacteria

HRE 664 possesses a broad antibacterial spectrum (Table 1), which includes both Staphylococci and Streptococci. The *in vitro* activity of HRE 664 against Staphylococci was clearly superior to all compared compounds. With an MIC 90% of 0.43 μg/ml, HRE 664 is also active against methicillin-resistant *Staphylococcus aureus* (MR*SA*). Against Enterococci HRE 664 also has a significantly higher activity than the other compounds (MIC 90%=5.04 μg/ml).

Only against *Pseudomonas aeruginosa* and *Pseudomonas maltophilia* HRE 664 shows very limited activity. However, HRE 664 shows excellent activity against other strains of *Pseudomonas*, the MIC 90% being 0.29 μg/ml.

HRE 664 is the most active compound against *Acinetobacter* sp.

The Enterobacteriaceae were highly susceptible to HRE 664. Ninety % of the *E. coli*, *Salmonella* sp., *Klebsiella* sp. and *Citrobacter* strains were inhibited at 1 μg/ml or less.

All strains of *Enterobacter* sp., *Serratia* sp., indole-negative and indole-positive *Proteus* sp. are inhibited at therapeutically relevant concentrations of 4~8 μg/ml.

Activity against Anaerobes

Table 2 shows the MICs of HRE 664 and three reference compounds against Gram-positive and

Table 1. *In vitro* activity of HRE 664 and compared compounds against aerobic and facultative anaerobic bacteria.

Strain/Antibiotic	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)
Staphylococcus sp. methicillin-sensitive n=40			
HRE 664	0.03	0.13	0.015~1.0
Ceftazidime	10.20	26.91	4.0~128.0
Cefotaxime	0.78	2.83	0.125~64.0
Piperacillin	2.83	27.86	0.5~>128.0
Staphylococcus aureus methicillin-resistant n=31			
HRE 664	0.07	0.43	0.015~16.0
Ceftazidime	45.26	>128.0	16.0~>128.0
Cefotaxime	5.19	59.71	0.5~>128.0
Piperacillin	83.0	>128.0	2.0~>128.0
Streptococcus sp. (serogroup A, B, C) n=40			
HRE 664	0.01	2.3	0.002~4.0
Ceftazidime	0.054	0.5	0.015~>128.0
Cefotaxime	<0.002	0.5	0.002~16.0
Piperacillin	0.026	0.5	<0.002~4.0
Streptococcus sp. (serogroup D) n=40			
HRE 664	2.4	5.04	1.0~8.0
Ceftazidime	>128.0	>128.0	32.0~>128.0
Cefotaxime	16.0	>128.0	0.062~>128.0
Piperacillin	3.36	16.0	2.0~32.0
Pseudomonas aeruginosa n=40			
HRE 664	68.84	123.41	8.0~>128.0
Ceftazidime	0.241	0.669	0.125~16.0
Cefotaxime	6.01	14.11	0.062~32.0
Piperacillin	2.38	6.22	0.062~16.0
Pseudomonas maltophilia n=11			
HRE 664	>128.0	>128.0	1.0~>128.0
Ceftazidime	8.0	>128.0	1.0~>128.0
Cefotaxime	71.83	>128.0	16.0~>128.0
Piperacillin	64.0	>128.0	0.031~>128.0
Pseudomonas sp. (other than *P. aeruginosa* or *P. maltophilia*) n=28			
HRE 664	0.003	0.29	<0.002~128.0
Ceftazidime	4.22	7.67	0.062~32.0
Cefotaxime	0.23	1.47	0.015~16.0
Piperacillin	0.054	2.14	<0.002~64.0
Acinetobacter sp. n=13			
HRE 664	0.71	2.96	0.25~4.0
Ceftazidime	0.794	3.65	0.125~128.0
Cefotaxime	1.68	47.4	0.5~64.0
Piperacillin	2.21	3.85	0.062~128.0

Table 1. (Continued)

Strain/Antibiotic	MIC_{50} (μg/ml)	MIC_{80} (μg/ml)	Range (μg/ml)
Escherichia coli			
n=40			
HRE 664	0.21	0.5	0.062~1.0
Ceftazidime	0.051	0.125	0.031~0.5
Cefotaxime	0.004	0.02	0.002~0.062
Piperacillin	0.41	>128.0	0.015~>128.0
Citrobacter sp.			
n=40			
HRE 664	0.42	0.9	0.25~8.0
Ceftazidime	0.202	32.0	0.031~>128.0
Cefotaxime	0.17	25.4	<0.002~128.0
Piperacillin	1.699	32.0	0.5~>128.0
Serratia sp.			
n=40			
HRE 664	1.67	3.23	2.0~4.0
Ceftazidime	0.029	0.057	0.008~0.125
Cefotaxime	0.36	1.26	0.25~4.0
Piperacillin	1.834	>128.0	0.5~>128.0
Salmonella sp.			
n=40			
HRE 664	0.18	0.41	0.125~1.0
Ceftazidime	0.021	0.062	<0.002~0.25
Cefotaxime	0.01	0.04	<0.002~0.125
Piperacillin	0.426	1.189	<0.002~4.0
Klebsiella sp.			
n=40			
HRE 664	0.33	1.0	0.25~4.0
Ceftazidime	0.02	0.099	0.004~0.5
Cefotaxime	0.01	0.18	<0.002~1.0
Piperacillin	2.94	128.0	0.5~>128.0
Enterobacter sp.			
n=40			
HRE 664	0.78	2.0	0.25~8.0
Ceftazidime	0.091	5.66	0.015~128.0
Cefotaxime	0.08	4.0	<0.002~128.0
Piperacillin	1.287	16.0	0.125~>128.0
Proteus sp.			
indole-negative			
n=30			
HRE 664	0.583	1.641	0.25~4.0
Ceftazidime	0.086	0.177	<0.002~0.25
Cefotaxime	<0.002	0.006	<0.002~0.015
Piperacillin	0.474	8.0	<0.002~>128.0
Proteus sp.			
indole-positive			
n=30			
HRE 664	1.0	2.38	0.5~4.0
Ceftazidime	0.088	2.0	0.015~8.0
Cefotaxime	0.315	2.73	<0.002~4.0
Piperacillin	0.66	22.63	0.5~>128.0

Table 2. *In vitro* activity against obligate anaerobes.

Strain	No.	MIC (μg/ml)			
		HRE 664	Metronidazole	Cefotaxime	Cefoxitin
Bacteroides fragilis	312*	0.125	0.78	50.0	1.56
	679*	<0.01	2.5	>100	6.25
	960*	0.156	0.39	100	3.125
	1313	0.313	0.19	12.5	12.5
	1367	0.625	1.25	1.563	6.25
	2423	0.156	0.313	3.125	12.5
	2458	0.078	0.625	3.125	25.0
	5436	0.625	0.625	1.563	12.5
	17390	0.313	0.391	3.125	3.125
	18125	0.25	0.39	3.125	6.25
	18206	0.039	0.625	1.563	6.25
	19016	0.625	0.78	6.25	6.25
	25285	<0.01	0.625	12.5	6.25
B. ovatus	103*	0.125	0.78	50.0	6.25
B. vulgatus	1446*	0.125	0.08	50.0	3.125
B. thetaiotaomicron	123*	0.125	0.39	25.0	25.0
	1428*	0.625	0.78	50.0	25.0
	1445*	0.625	0.78	50.0	50.0
B. distasonis	1366	0.625	0.39	0.195	1.563
	5523	0.039	1.25	0.781	12.5
B. ruminicola	2457	0.625	1.25	1.563	6.25
Fusobacterium varium	3085*	0.313	0.19	25.0	50.0
	5262	0.156	0.39	0.391	6.25
	8501	0.078	0.313	3.125	3.125
Sphaerophorus freundi	1369*	0.156	0.195	25.0	3.125
S. necrophorus	1375	0.313	0.625	3.125	3.125
Peptostreptococcus anaerobius	932	0.019	0.78	0.391	0.781
Peptococcus anaerobius	1449	0.019	ND	<0.098	0.195
Propionibacterium acnes	6919	0.019	>100	0.195	0.195
	6922	0.019	>100	0.195	0.195
Clostridium tetani	1004	0.25	0.04	6.25	0.195
	19406	0.313	<0.1	6.25	0.391
C. perfringens	194	0.313	0.78	0.781	0.781
C. septicum	184	0.019	ND	0.195	0.195
C. sporogenes	19404	0.313	0.625	6.25	0.195

* β-Lactamase producer.
ND: Not determined.

Table 3. Influence of the inoculum size on the *in vitro* activity of HRE 664.

Strain	MIC (μg/ml)			
	10^{6} [b]	10^{5}	10^{4}	10^{3}
Escherichia coli 1507E	0.625	0.313	0.313	0.313
E. coli TEM[a]	0.625	0.313	0.313	0.313
Enterobacter cloacae 1321E	0.625	0.625	0.625	0.625
E. cloacae P99[a]	5	5	2.5	2.5
Staphylococcus aureus penicillin-sensitive	0.016	0.031	0.016	0.016
S. aureus[a] penicillin-resistant	0.031	0.016	0.016	0.016

[a] Strain producing β-lactamase.
[b] Inoculum size.

Gram-negative anaerobes. HRE 664 is the most active compound against nearly all strains tested—even when compared with metronidazole. The highest MIC determined for some *Bacteroides* strains is 0.625 μg/ml.

Influence of the Inoculum Size on the MIC

The MICs of HRE 664 determined with different sizes of inocula of various test organisms are listed in Table 3. From these values it can be deduced that the inoculum size has little or no effect on the *in vitro* activity of HRE 664 against β-lactamase producing or non-producing strains.

Table 4. Dependence of the *in vitro* antibacterial activity of HRE 664 on pH.

Strain	MIC (μg/ml)							
	pH 5.5	pH 6.0	pH 6.5	pH 7.0	pH 7.5	pH 8.0	pH 8.5	pH 9.0
Staphylococcus aureus methicillin-sensitive	<0.002	<0.002	0.008	0.015	0.062	0.125	0.250	0.250
Staphylococcus sp. methicillin-resistant	0.015	0.015	0.015	0.062	0.062	0.125	0.250	1.0
Streptococcus pyogenes serogroup A	0.008	0.008	0.015	0.015	0.031	0.062	0.125	0.125
S. faecium MD 8B	6.25	6.25	3.13	6.25	12.5	12.5	12.5	12.5
Escherichia coli TEM I	0.625	0.625	0.313	0.313	0.625	1.25	0.625	0.313
Salmonella typhimurium	0.625	1.25	0.625	0.625	0.625	0.625	1.25	0.625
Pseudomonas aeruginosa ATCC 9027	100	100	100	100	100	100	100	100

Fig. 2. Bactericidal activity of HRE 664 against *Escherichia coli* TEM I.
□ Control, △ 2-fold MIC, ○ MIC, ● 1/2 MIC.

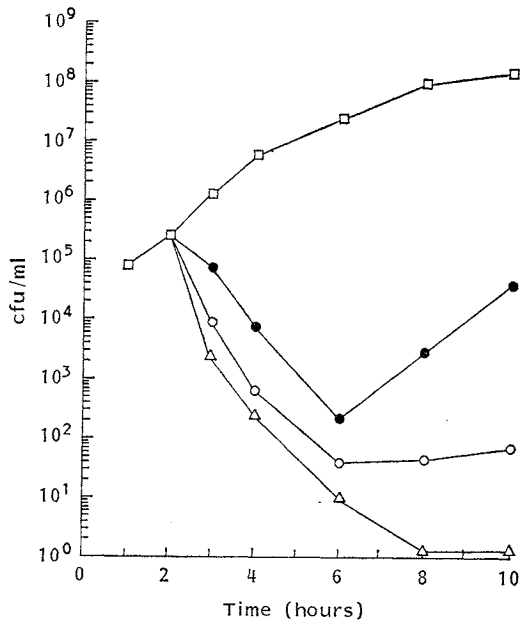


Fig. 3. Bactericidal activity of HRE 664 against *Staphylococcus aureus* SG 511.
□ Control, △ 2-fold MIC, ○ MIC, ● 1/2 MIC.

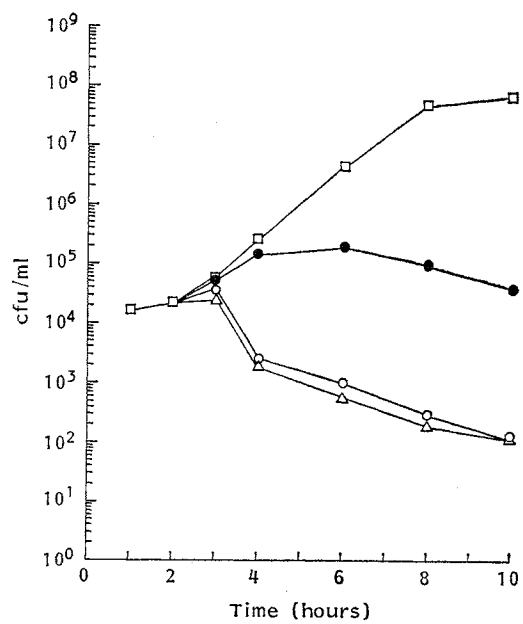


Table 5. β-Lactamase stability of HRE 664 and cefotaxime.

Enzyme from	Richmond class	Relative rate of hydrolysis*	
		HRE 664	Cefotaxime
Escherichia coli TEM I	III a	<1	<1
Enterobacter cloacae P99	I	<1	<1
Klebsiella sp. 1082E	IV c	<1	5.9
Citrobacter freundii J20	—	1.5	8.1
Bacteroides sp.	—	<1	22.0

* Cephaloridine=100.

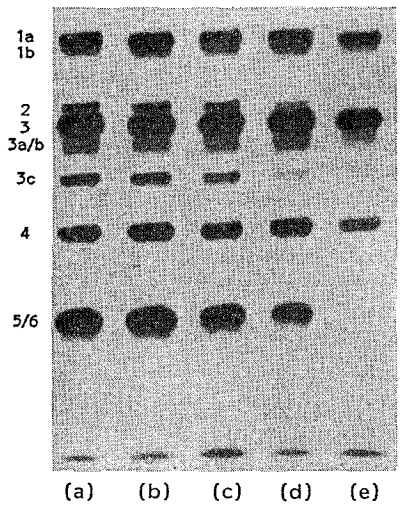
Effect of the pH of the Culture Medium on the MIC

From Table 4, it can be seen that the antibacterial activity of HRE 664 against the Gram-negative test strains and *Streptococcus faecium* is virtually unaffected by the pH of the test medium. The MICs determined between pH 5.5 and pH 9.0 differ only by one to two dilution steps which is in the range of variation of the assay method.

However, for the other test strains marked differences are evident in the MICs of 4 (*Streptococcus pyogenes* A 77), 6 (*Staphylococcus* sp. E 710) and more than 7 dilution steps (*Staphylococcus aureus* SG 511). HRE 664 is far more active at an acidic pH than at basic pH values against these strains.

Fig. 4. Binding of HRE 664 to the penicillin-binding proteins from *E. coli* K-12.

Control (a), HRE 664 0.001 μg/ml (b), 0.01 μg/ml (c), 0.1 μg/ml (d), 1 μg/ml (e).



Bactericidal Activity

Figs. 2 and 3 show the bactericidal activity of HRE 664 against *E. coli* TEM I and *S. aureus* SG 511. The slope of the curves in Fig. 2 demonstrates that the number of surviving *E. coli* cells decreases steadily after the addition of HRE 664 in concentrations corresponding to the MIC and 2-fold MIC. At 1/2 MIC, HRE 664 induced a 2-hour retardation in bacterial growth. With *S. aureus* SG 511 (Fig. 3), the addition of HRE 664 at one and two times the MIC, the number of cfu in the culture increased slightly for 1 hour but subsequently decreased rapidly. At sub-inhibitory concentrations (1/2 MIC), *S. aureus* SG 511 was not inhibited by HRE 664.

β-Lactamase Stability

Table 5 shows the relative rates of hydrolysis of HRE 664 and cefotaxime. The data indicate that HRE 664 is extremely stable against all β-lactamases tested. No significant hydrolysis could be detected for HRE 664 — even against chromosomally coded enzymes which hydrolyze cefotaxime rather rapidly.

Affinity for Penicillin-binding Proteins (PBPs)

As shown in Fig. 4, HRE 664 binds preferentially to the PBP 2, 3c, 5 and 6. Binding to PBP 1 can only be seen at higher concentrations (1 μg/ml) of HRE 664. In the *in vitro* bactericidal assay,

binding to PBP 2 causes the formation of round shaped forms which lyze rapidly at the MIC or above.

Discussion

In contrast to modern cephalosporins and penicillins, the penem antibiotics have shown a unique antibacterial spectrum which includes anaerobic strains as well as Gram-positive and most Gram-negative pathogens[1~3]. These properties can also be found with the new penem HRE 664. The results presented here clearly demonstrate that HRE 664 is much more potent against Gram-positive strains than the compared cephalosporins ceftazidime and cefotaxime. However, slightly higher MICs of HRE 664 have been determined for most Gram-negative pathogens, which nevertheless should allow successful therapy of infections caused by these organisms.

In contrast to other β-lactams, HRE 664 also exhibits marked activity against Gram-positive and Gram-negative anaerobic bacteria. Its activity against these strains is clearly superior to that of cefoxitin and metronidazole-agents, which are normally used in anaerobic infections.

Since initial results also show excellent chemotherapeutic activity *in vivo* in experimental infections, we think it would be worthwhile to continue the evaluation of HRE 664.

References

1) BROWN, R. M.; R. WISE & J. M. ANDREWS: Sch 29482—a novel penem antibiotic: an *in-vitro* comparison of its activity with other β-lactams. J. Antimicrob. Chemother. 9 (Suppl. C): 17~23, 1982
2) WISE, R.; J. M. ANDREWS & G. DANKS: Comparison of in vitro activity of FCE 22101, a new penem, with those of other β-lactam antibiotics. Antimicrob. Agents Chemother. 24: 909~914, 1983
3) NEU, H. C.; N. X. CHIN & P. LABTHAVIKUL: *In-vitro* activity and β-lactamase stability and inhibitory properties of a new penem antibiotic, Sch 34343. J. Antimicrob. Chemother. 15 (Suppl. C): 25~37, 1985
4) SEIBERT, G.; M. LIMBERT, I. WINKLER & T. DICK: The antibacterial activity *in vitro* and β-lactamase stability of the new cephalosporin HR 810 in comparison with five other cephalosporins and two aminoglycosides. Infection 11: 275~279, 1983
5) SCHWARZ, U.; K. SEEGER, F. WENGENMAYER & H. STRECKER: Penicillin-binding proteins of *Escherichia coli* identified with a ^{125}I-derivative of ampicillin. FEMS Microbiol. Lett. 10: 107~109, 1981