HRE 664, A NEW PARENTERAL PENEM

II. EVALUATION OF THE PHARMACOKINETIC BEHAVIOR AND THE CHEMOTHERAPEUTIC ACTIVITY IN ANIMALS

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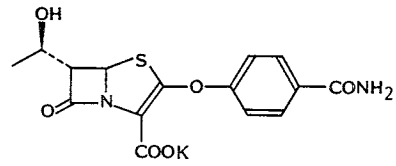
The pharmacokinetic and chemotherapeutic properties of the new penem antibiotic HRE 664 (Fig. 1) were evaluated in experimental animals. High and sustained blood and serum levels were achieved following parenteral injection in mice, rats, dogs and monkeys. Half-lives ranged from 27 to 40 minutes in the various species tested. The antibiotic was well distributed in the rodents and penetrated well into tissues and body fluids. At 30 minutes after subcutaneous administration to mice (50 mg/kg), concentrations of between 12.4 and 35.9 μg/g were measured in the lungs, liver, heart and kidneys, that is 33~95% of the corresponding level in murine blood (37.7 μg/ml).

In experimentally induced infections in mice, HRE 664 displayed good chemotherapeutic activity particularly against septicemias caused by methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* strains and on abscess formation induced by *Bacteroides fragilis*. Most of the cephalosporins and other β-lactam antibiotics exhibited low efficacy against these strains of bacteria.

HRE 664 (potassium 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) is a new penem antibiotic with a broad spectrum of antibacterial activity *in vitro*. The HRE 664 spectrum includes Enterobacteriaceae, methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* strains as well as obligate Gram-positive and Gram-negative anaerobes[1]. The compound is highly resistant to hydrolysis by various types of β-lactamases.

The purpose of this study was to evaluate the pharmacokinetic behavior of the new compound in laboratory animals and compare the chemotherapeutic efficacy of HRE 664 in experimental infections with that of broad spectrum β-lactam antibiotics, such as cefotaxime, in experimental infections with *Bacteroides fragilis* also with cefoxitin, latamoxef and metronidazole.

Fig. 1. Chemical structure of HRE 664.



Materials and Methods

Antibiotics

HRE 664 was synthesized at Hoechst Pharmaceutical Research Laboratories, Milton Keynes, Great Britain. Cefotaxime was a commercial preparation from Hoechst/Glaxo, Frankfurt, FRG.

Cefoxitin, latamoxef and metronidazole were obtained from a commercial source. The antibiotics were dissolved in sterile water just before use.

Concentrations of HRE 664 in Blood and Urine

Groups of six NMRI albino mice and six Sprague-Dawley rats weighing 18~22 g and 190~210 g, respectively, were injected intravenously *via* a caudal vein with 10, 20 or 50 mg/kg. At various times between 5 and 180 minutes after dosing, 10 μl of blood were removed from a cut on the tip of the tail by means of capillary tubes (Wiretrol, mfr.: Drummond, Broomall, U.S.A.) wetted with sodium citrate and stored at 4°C until further processing.

Three male beagle dogs weighing 17.5±1.3 kg and three male monkeys (*Macaca arctoides*) weighing 5.9±0.8 kg were dosed intravenously with 20 mg/kg *via* the *vena cephalica antibrachii*. Blood samples were withdrawn from the cephalic vein of the opposite leg. The serum was separated and stored at −20°C until being assayed for serum antibiotic levels.

Urine was collected from the mice, rats and monkeys in metabolic cages, from the dogs by catheterization of the bladder.

Concentrations in Tissues and Tissue Fluid

30 and 60 minutes after subcutaneous dosing with 50 mg/kg, groups of ten NMRI mice were killed by exsanguination and the heart, liver, lungs, kidneys and thigh muscle were removed. The various tissue samples from the individual mice were examined for their antibiotic concentration separately.

A pair of sterile multi-perforated plastic capsules (Eppendorf micro test tubes) were implanted into the *subcutis* on each side of the back of ten Wistar rats weighing 150±10 g[2]. After 14 days, when tissue cages were filled with interstitial fluid the rats were given an intramuscular injection of 20 mg/kg. At certain times after the injection, 0.1 ml of tissue fluid was obtained from the tissue cages by percutaneous aspiration with a fine needle (No. 18). Blood samples were taken simultaneously from a caudal vein as described above.

Bioassay of HRE 664

Concentrations in blood, urine, serum, tissue fluid and tissue samples were determined microbiologically by the agar diffusion test. The culture medium was Mueller-Hinton broth with 1.9% agar and 10% sheep's blood[3]. *Streptococcus pyogenes* A77 was used as the indicator organism. The standard solution and diluent were prepared with the following: Blood or serum for determining the blood/serum level; urine for determining the urine concentrations; phosphate buffer (pH 6.88, 0.025 M) for determining the tissue fluid; homogenates of untreated tissues and organs (tissue - buffer ratio, 1 : 5) for determining the concentrations in organs and tissues. Depending on the origin of the samples tested, the detection limits of HRE 664 ranged from 0.4 to 1.6 μg/ml. Blood, serum and tissue fluid concentrations were calculated by regression analysis with the help of the standard curves in which the logarithms of concentrations were proportional to the areas of the inhibition zones. Following intramuscular administration of HRE 664 to rats, blood and tissue fluid concentration-time data were fitted to a one-compartment model, whereas after intravenous administration, the distribution of the compound in test animals followed an open two-compartment model. Based on the assay results, the pharmacokinetic parameters were calculated as described by WAGNER[4].

The individual tissue samples were contaminated with varying amounts of blood. In order to calculate the actual extravascular concentration of the antibiotic, the haemoglobin content was determined and used as a parameter for the amount of blood in the tissue samples[3].

Protection Test in Mice

Protection studies in NMRI mice weighing 18~22 g were carried out as described[5]. Eight Gram-positive and nine Gram-negative test organisms were used in the experiments. The strains were maintained as suspension in 15% skimmed milk in liquid nitrogen.

In the tests, mice were infected intraperitoneally with 0.3 ml of bacterial suspension in 5% hog gastric mucin. Depending on the infecting organism, the challenge inoculum contained 4 to 100 times the LD_{100} of the pathogens. A group of ten untreated animals was always used as controls. They died between 6 and 24 hours after being infected, depending on the type of bacteria with which they

were infected.

Ten mice were used for each of the serial 2-fold dose concentrations of HRE 664 or the reference compound cefotaxime. Treatment was subcutaneous — immediately and 4 hours after infection. The median effective dose (ED_{50} mg/kg/total dose) was calculated by probit analysis from the number of mice surviving on day 10. HRE 664 and the reference compounds were evaluated in parallel against each test organism.

Anaerobic Infections in Tissue Cages

Multi-perforated tissue cages implanted into the subcutis of rats 14 days previously were injected with 0.1 ml of a 48-hour culture of *Bacteroides fragilis* 960, containing 1.3×10^{-7} cfu/ml. Intramuscular treatment was initiated 24 hours after infection of the tissue cages and was continued twice daily for 4 days. The dosages were 20 mg/kg body weight of HRE 664 or the reference compounds cefotaxime, cefoxitin and latamoxef. Growth of *B. fragilis* 960 in the tissue cages or reduction of the organism by antibacterial treatment was monitored by determining the number of viable cells in 0.1 ml tissue fluid sample prior to first administration of the drugs and at 48, 72, 96 and 104 hours after infection.

Anaerobic Subcutaneous Abscesses in Mice

hrCH3 mice (hairless mice) of both sexes weighing 18~23 g were infected with *B. fragilis* 18125 in semisolid medium (0.25% agar) according to the abscess model of WALKER and WILKINS[6]. Therapy was initiated 1 hour after infection by subcutaneous injection of 50 mg/kg HRE 664, cefoxitin, latamoxef or cefotaxime on the opposite side to the abscesses and continued twice daily for three days. The total dosage was 300 mg/kg of each compound. Metronidazole was given orally in a dose of 50 mg/kg. Ten animals were used in each group. At day 4 after the infection, the number of cfu/g abscess was determined.

Results

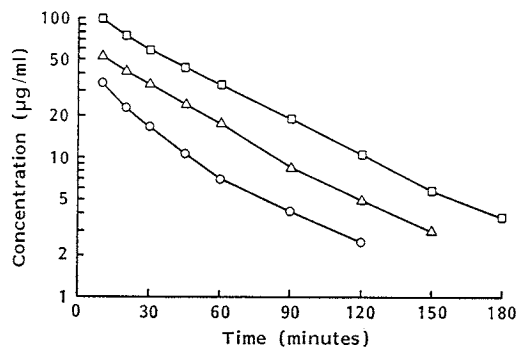
Pharmacokinetic Properties of HRE 664

The mean HRE 664 concentrations in the blood and serum of rodents, dogs and monkeys are presented in Figs 2~7. The derived pharmacokinetic parameters are summarized in Tables 1 and 2.

Mice

Blood levels of HRE 664 in mice following single intravenous injections of 10, 20 or 50 mg/kg are shown in Fig. 2. Initial blood concentrations (C_P^0) of 52.7, 74.1 and 240.0 μg/ml were obtained rapidly after dosing. The elimination half-life was about 0.6 hour and the areas under the concentration time curves (AUCs) amounted to 25.7, 51.8 and 106 μg·hours/ml. With respect to the AUCs, HRE 664 showed a linear dose response following intravenous injection. Low HRE 664 concentrations above the detection limit could still be detected in the murine blood even at 2 to 3 hours after administration. The percentage of the compound recovered in urine within 18 hours of dosing was 36.3±8.0%.

Fig. 2. Mean blood concentrations of HRE 664 in mice after a single intravenous injection.
□ 50 mg/kg, △ 20 mg/kg, ○ 10 mg/kg.



Rats

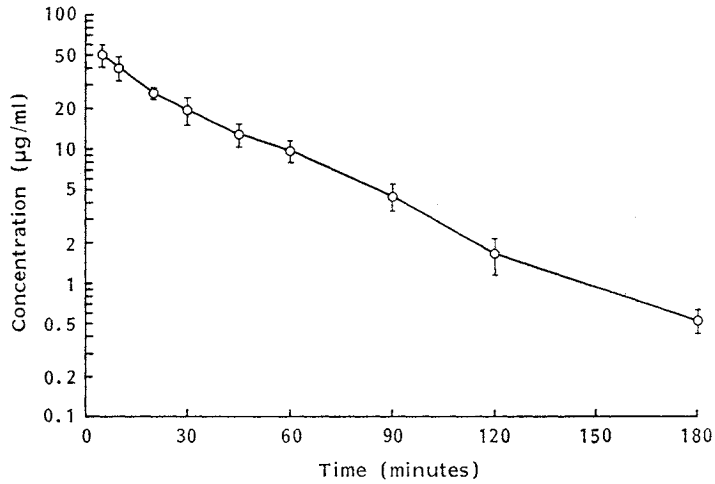
The fictive concentration at time t=0 calculated for rats after 10 mg/kg was 71.5±20.2 μg/ml. HRE 664 had a $t_{1/2\beta}$ of 0.59±0.07 hour and AUC was 30.5±4.8 μg·hours/ml. Three hours after dosing a mean value of 0.5 μg/ml

Table 1. Pharmacokinetic parameters (mean±SD) of HRE 664 in rodents after a single intravenous dose of 10, 20 or 50 mg/kg.

Parameter[a]	Mice[b]			Rats[b]
	10	20	50	10
Route	iv	iv	iv	iv
C_P° (μg/ml)	52.7±7.1	74.1±8.0	240.0±48.8	71.5±20.2
$t_{1/2\alpha}$ (hour)	0.06±0.04	0.06±0.02	0.06±0.02	0.11±0.05
$t_{1/2\beta}$ (hour)	0.54±0.28	0.57±1.7	0.67±0.09	0.59±0.07
$AUC_{0\sim\infty}$ (μg·hours/ml)	25.7±4.9	51.8±4.2	106.0±10.4	30.5±4.8
ΔD_{ss} (liter/kg)	0.21±0.03	0.3±0.04	0.41±0.05	0.21±0.05
Cl_{Tot} (ml/minute)	0.13±0.02	0.13±0.01	0.16±0.02	1.12±0.17
Cl_{Ren} (ml/minute)	0.05±0.01	0.05±0.01	0.06±0.01	0.44±0.09
$UR_{0\sim18\,hours}$ (%)		36.3±8.0		39.5±8.3

[a] C_P°: Fictive concentration at time t=0, $t_{1/2\alpha}$: half-life in distribution phase, $t_{1/2\beta}$: half-life in elimination phase, AUC: area under concentration *versus* time curve, ΔD_{ss}: steady-state distribution volume per body weight, Cl_{Tot}: total body clearance, Cl_{Ren}: renal clearance, UR: urinary recovery.
[b] Dose (mg/kg).

Fig. 3. Mean blood concentrations of HRE 664 in rats after a single intravenous injection (10 mg/kg). $t_{1/2}$=29 minutes.



was still measured in the blood. 39.5±8.3% of the administered dose was excreted in the urine (Table 1 and Fig. 3).

Dogs

Following the intravenous injection of 20 mg/kg HRE 664, the serum level decreased with a $t_{1/2\beta}$ of 0.66±0.10 hour to a concentration of 1.0 μg/ml at 6 hours. The AUC was calculated as 100.6±33.1 μg·hours/ml. Urine recovery during the period between 0 and 8 hours amounted to 29.7±5.1% of the given dose (Table 2 and Fig. 4).

Table 2. Pharmacokinetics of HRE 664 in dogs and monkeys after a single intravenous dose of 20 mg/kg.

Parameter[a]	Dogs	Monkeys
$t_{1/2\alpha}$ (hour)	0.06±0.02	0.06±0.03
$t_{1/2\beta}$ (hour)	0.66±0.10	0.44±0.07
$AUC_{0\sim\infty}$ (μg·hours/ml)	100.6±33.1	60.6±6.0
ΔD_{ss} (liter/kg)	0.14±0.07	0.12±0.02
Cl_{Tot} (ml/minute)	62.1±19.5	32.8±3.7
Cl_{Ren} (ml/minute)	19.1±8.8	4.5±1.9
$UR_{0\sim8\,hours}$ (%)	29.7±5.1	13.4±4.0

[a] For abbreviations see Table 1.

Fig. 4. Mean serum concentrations of HRE 664 in dogs after a single intravenous injection (20 mg/kg). $t_{1/2}$=40 minutes.

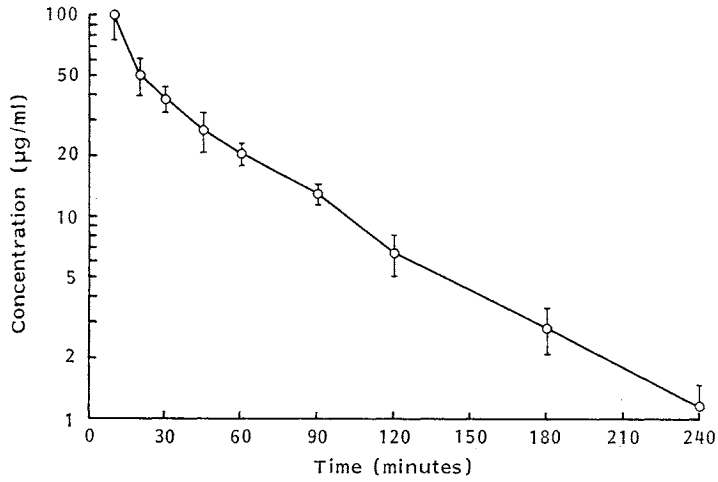
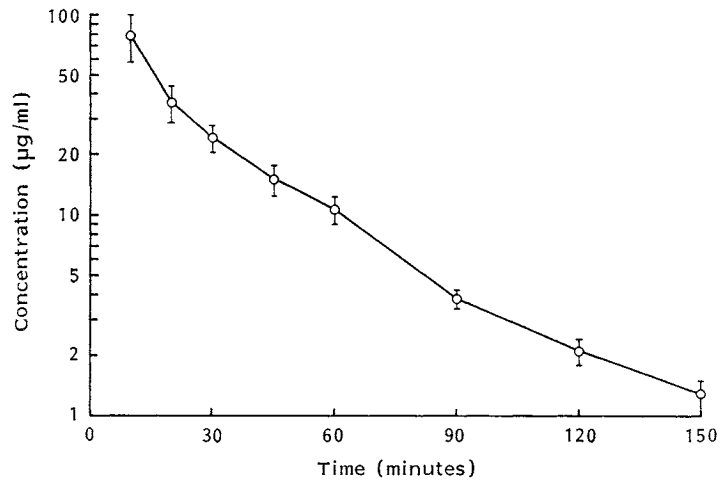


Fig. 5. Mean serum concentrations of HRE 664 in monkeys after a single intravenous injection (20 mg/kg). $t_{1/2}$=27 minutes.



Monkeys

In *Macaca arctoides* monkey serum levels and pharmacokinetic parameters were somewhat lower than in the other species tested. $t_{1/2\beta}$ was 0.44±0.07 hour, AUC 60.6±6.0 μg·hours/ml and urine recovery 13.4±4.0%. Serum concentrations were detected up to 150 minutes after administration, the detection limit being 0.8 μg/ml (Table 2 and Fig. 5).

Tissue Concentrations in Rodents

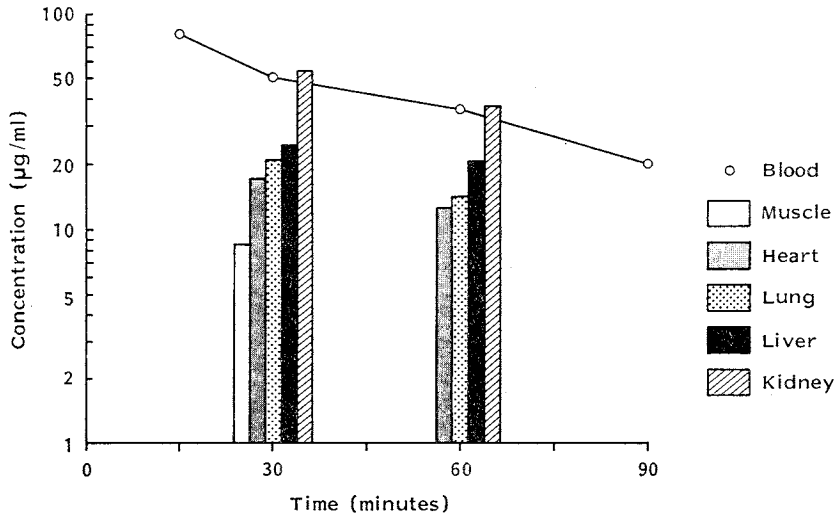
30 and 60 minutes after subcutaneous administration of 50 mg/kg HRE 664 to NMRI mice, blood levels of 50.0±2.8 and 37.7±1.6 μg/ml were measured respectively (Table 3 and Fig. 6). With 53.7+10.3 and 35.9±4.2 μg/g at 30 and 60 minutes, the concentrations in kidneys reached the same level as those in the blood. Concentrations of between 12.4±1.7 and 24.2±5.0 μg/g were also measured in the lungs, liver and heart at 60 minutes after injection, that is 33~95% of the correspond-

Table 3. Blood and tissue concentrations of HRE 664 in mice after a single subcutaneous injection of 50 mg/kg.

Tissue	Median concentrations±SD (μg/ml or μg/g)		% of blood concentrations	
	0.5 hour	1.0 hour	0.5 hour	1.0 hour
Blood	50.0±2.8	37.7±1.6	100	100
Heart	17.0±1.9	12.4±1.7	34	33
Lung	20.7±1.2	13.8±2.2	41	37
Liver	24.2±4.9	20.2±6.5	48	54
Kidney	53.7±10.3	35.9±4.2	107	95
Muscle	8.4±3.2	<3.1	17	<8.2
Pus (abscess)	ND	13.3±3.1	ND	47

ND: Not determined.

Fig. 6. Blood and tissue concentrations of HRE 664 in mice after a single subcutaneous injection (50 mg/kg).



ing level in murine blood. A concentration of 8.4±3.2 μg/g was detected in thigh muscle at 30 minutes but this had fallen below the detection limit (0.8 μg/g) at 60 minutes after administration.

HRE 664 showed good penetration into subcutaneous abscesses in hrCH3 mice infected by subcutaneous injection of *Bacteroides fragilis* 18125. One hour after administration of 50 mg/kg, a pus concentration of 13.3±3.1 μg/g was found, that is 47% of the corresponding drug concentration in blood (28.0±3.0 μg/ml).

Rats implanted with multi-perforated tissue cages were infected percutaneously with *B. fragilis* 960. Twenty-four hours later a single intramuscular dose of 20 mg/kg HRE 664 was given. The blood and tissue fluid concentrations are shown in Table 4 and Fig. 7. High but short blood levels (Cmax 40.9±11.7 μg/ml, $t_{1/2\beta}$ 0.8±0.1 hour) and low (Cmax 4.4±1.2 μg/ml) but sustained levels ($t_{1/2\beta}$ 4.0± 2.3 hours) were measured in tissue cage fluid (Table 4 and Fig. 7).

Chemotherapeutic Activity in Experimentally Induced Infections

Protection Test in Mice

The results of the sensitivity test of the infecting organism and of the protection tests in mice are

Table 4. Pharmacokinetic parameters (mean±SD) of HRE 664 after a single intramuscular dose of 20 mg/kg in rats with infected tissue cages.

Parameter	Blood	Tissue cage fluid
tmax (hours)	0.5±0.2	1.4±0.5
Cmax (μg/ml)	40.9±11.7	4.4±1.2
$t_{1/2\beta}$ (hours)	0.8±0.1	4.0±2.3
$AUC_{0\sim\infty}$ (μg·hours/ml)	61.4±7.7	28.4±5.8

tmax: Time of maximum concentration, Cmax: maximum concentration, $t_{1/2\beta}$: half-life in elimination phase, AUC: area under concentration *versus* time curve.

Fig. 7. Mean blood and tissue cage concentrations of HRE 664 in rats after a single intramuscular injection (20 mg/kg).
○ Blood, □ tissue cage.

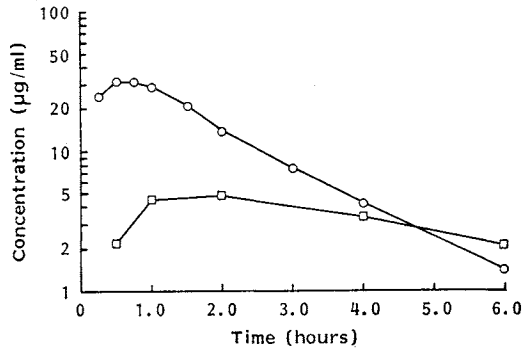


Table 5. Comparative activity of HRE 664 and cefotaxime in protection tests in mice.

Test strain	HRE 664		Cefotaxime	
	MIC (μg/ml)	ED_{50} (mg/kg)	MIC (μg/ml)	ED_{50} (mg/kg)
Staphylococcus aureus Giorgio[a]	0.05	0.73	2.0	4.74
S. aureus SG 511[a]	0.05	0.55	1.0	15.52
S. aureus E 703[b]	0.25	37.77	2.0	>800.0
S. aureus E 710[b]	0.5	2.5	8.0	132.4
S. aureus 31153[b]	0.05	1.62	4.0	57.16
Streptococcus pyogenes A77	0.05	0.9	<0.002	0.17
Streptococcus sp. EDER D	8.0	59.61	>128.0	>800.0
Streptococcus sp. FO 3 D	4.0	69.15	>128.0	>800.0
Escherichia coli 078	0.2	2.84	<0.002	0.04
Klebsiella pneumoniae DT-S	0.39	15.0	<0.002	0.78
K. aerogenes 1082E[c]	0.25	6.88	2.0	19.76
Salmonella typhimurium MZ II	0.39	4.26	0.03	0.07
Citrobacter freundii GN346[c]	1.0	42.75	32.0	51.0
Enterobacter cloacae M 417	0.25	10.79	0.015	0.38
Serratia marcescens M 378	3.13	11.28	0.39	3.03
Proteus mirabilis ATCC 14273	1.56	32.32	0.01	1.52
Morganella morganii 939	2.0	25.0	0.125	3.72

[a] Methicillin-sensitive, [b] methicillin-resistant, [c] β-lactamase producer.

summarized in Table 5. In this model, the chemotherapeutic efficacy of HRE 664 was compared to that of the cephalosporin cefotaxime.

As shown in the tables, the high *in vitro* activity of HRE 664 against methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) *Staphylococcus aureus* strains was reflected by good *in vivo* activity against experimental infections. The ED_{50} values of HRE 664 were 0.55 and 0.73 mg/kg in the case of MSSA infections and ranged from 1.62 to 37.77 mg/kg in septicemias caused by MRSA strains. The penem was thus six to fifty times more active than the reference compound cefotaxime.

HRE 664 even showed activity against Enterococci — strains which were resistant to cefotaxime and other cephalosporin derivatives. In the case of infections caused by *Streptococcus pyogenes* A77 and β-lactamase-non-producing Enterobacteriaceae strains, however, cefotaxime displayed a distinctly

Table 6. Chemotherapeutic activity of HRE 664 and reference compounds in *Bacteroides fragilis* abscesses in mice.

Total dose: 300 mg/kg sc, metronidazole: 300 mg/kg po.

Reduction of viable bacteria[a]	HRE 664	Cefotaxime	Cefoxitin	Latamoxef	Metronidazole
No abscess formation	5/10	1/10	0/10	1/9	2/9
2~5 $\log_{10}$-fold	2/10	0/10	0/10	1/9	7/9
<2 $\log_{10}$-fold	3/10	9/10	10/10	7/9	0/9

[a] Compared to the untreated controls (n=10, $\bar{x}\pm SD=9.4\pm0.2$ cfu/g).

higher efficacy *in vitro* and *in vivo* than the penem antibiotic. However, three times lower or similar doses of HRE 664 had to be given for the eradication of septicemia caused by *Klebsiella aerogenes* 1082E and *Citrobacter freundii* GN346 both of which produced chromosomally coded β-lactamase.

Localized Infections with *Bacteroides fragilis*

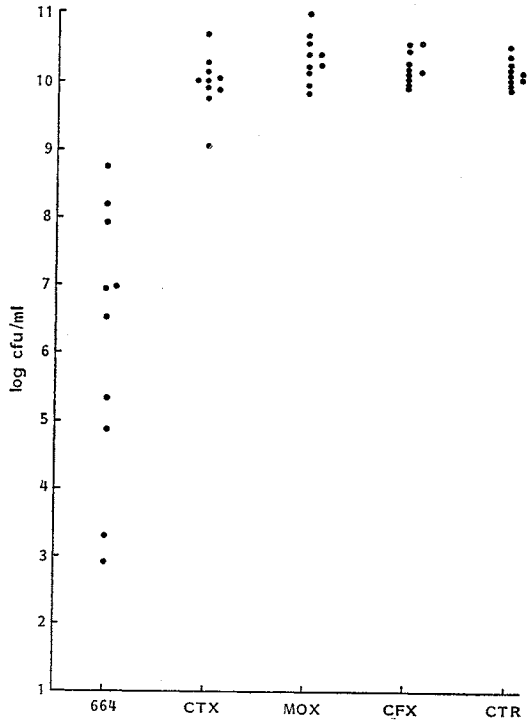
Table 6 presents the results of the treatment of abscesses due to *Bacteroides fragilis* 18125 in mice with an administration twice daily for 4 days. Therapy with six doses of 50 mg/kg HRE 664 resulted in the absence of abscesses in five out of ten mice and in an 1~4 $\log_{10}$-fold decrease in cfu/g in the abscesses of five mice. In mice treated with the reference compounds, however, only metronidazole showed good activity, *i.e.* 4~6 $\log_{10}$-fold decrease in the count of viable bacteria, while latamoxef, cefotaxime and cefoxitin were only slightly active or not active at all. All but one mouse of the cefotaxime group and the latamoxef group had abscesses when killed.

Compared to the untreated controls a marked decrease of viable bacteria was also observed after HRE 664 in the rats with tissue cages infected with *B. fragilis* 960. Cefotaxime, cefoxitin and latamoxef displayed no activity in this model (Fig. 8).

Fig. 8. Viable bacteria in tissue cages 104 hours after infection with *Bacteroides fragilis* in rats.

Intramuscular treatment with 20 mg/kg body weight HRE 664, cefotaxime (CTX), cefoxitin (CFX) or latamoxef (MOX) twice daily (total dosage: 140 mg/kg). Controls (CTR).

Limit of detection: $\log_{10}$ 3.0 cfu/ml.



Discussion

HRE 664, a new penem antibiotic, exhibits a favorable pharmacokinetic profile in experimental animals. High and sustained levels are achieved after parenteral administration. Peak levels, half-lives and areas under the curve were distinctly higher than those of two other penem antibiotics, FCE 22101 and Sch 34343, described in the literature[7~10]. Good penetration of HRE 664 from the blood stream into various tissues and body fluids was observed, and tissue concentrations up to 100% of the corresponding blood values were obtained.

These comparatively good pharmacokinetic properties were paralleled by high efficacy in experi-

mentally induced systemic or localized infections. We have to emphasize the high efficacy of HRE 664 against infections caused by those pathogens characterized by resistance or low sensitivity to various other β-lactam antibiotics, *i.e.* its high activity against Staphylococci, Enterococci, β-lactamase-producing Enterobacteriaceae and anaerobes. Of significance are the observations that HRE 664 therapy of the localized models with *B. fragilis* resulted in a distinctly higher killing of bacteria than treatment with cefotaxime, cefoxitin and latamoxef.

WELLS *et al.*[11] and JOINER *et al.*[12] described some bactericidal effects of Sch 34343, clindamycin and cefoxitin on bacterial cell count in *B. fragilis* abscesses and efficacy on abscess development, but both authors needed unrealistically high amounts of antibiotic (up to 10 g of total dose) to obtain such results. Although it is impossible to compare results obtained by using animal models with different infecting strains and treatment schedules in different laboratories, it is noteworthy that relatively low dosages of HRE 664 (up to 300 mg of total dose) were effective in localized infections caused by *B. fragilis* strains.

This study in laboratory animals demonstrated that HRE 664 is a β-lactam antibiotic which favorably combines high activity against various pathogens, good pharmacokinetic properties and bactericidal activity at the site of infection. The results presented in this paper encourage to the further development of this compound and further investigations.

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