COMPARATIVE EFFECTS OF CEFPIROME (HR 810) AND OTHER CEPHALOSPORINS ON EXPERIMENTALLY INDUCED PNEUMONIA IN MICE

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The chemotherapeutic efficacy of cefpirome (HR 810), a new polar aminothiazolyl-cephalosporin and that of ceftazidime, cefotaxime, cefoperazone, latamoxef and cefodizime were examined against experimental pneumonia caused by *Klebsiella pneumoniae* DT-S in mice. When compared in terms of MIC values against the infecting organism and the pharmacokinetic pattern, cefpirome showed equal activity and a similar pharmacokinetic behavior to ceftazidime and cefotaxime in mice. Trials to assess the bactericidial activity *in vivo*, however, showed that cefpirome displayed a more marked bactericidal effect in pneumonic mice than the other cephalosporins tested. Only cefodizime, a cephalosporin with extremely high and prolonged blood and tissue levels in experimental animals exerted chemotherapeutic effects similar to cefpirome. After cefpirome or cefodizime medication (50 mg/kg), the viable counts in the lungs of experimental animals fell steadily to 1/10,000 of the pretreatment level and, in contrast to the reference compounds, no regrowth of the challenge organisms could be observed with both drugs. Moreover, with ED_{50}s ranging from 1.1 to 59.1 mg/kg in treatment studies, cefpirome as well as cefodizime were two to ten times more effective than ceftazidime and cefotaxime, whereas cefoperazone and latamoxef were considerably less effective.

Cefpirome (3-((2,3-cyclopenteno-1-pyridium)methyl)-7-(2-*syn*-methoximino-2-(2-aminothiazol-4-yl)acetamido)ceph-3-em-4-carboxylate, HR 810) is a new semi-synthetic parenteral cephalosporin antibiotic with a broad spectrum of antibacterial activity *in vitro* and *in vivo*. The cefpirome spectrum includes *Enterobacteriaceae*, strains of the genera *Pseudomonas* and *Staphylococcus* as well as streptococci of the sero-group D[1~4]. It is also highly effective in controlling systemic and localized infections caused by these strains in experimental animals[5]. Investigations on the pharmacokinetic behavior of cefpirome showed high and sustained blood levels and good diffusibility into various tissues of experimental animals after sc, iv and im injections[6]. The purpose of this study was to determine the chemotherapeutic efficacy of cefpirome in experimentally induced lung infections in mice as compared with that of ceftazidime, cefotaxime, cefoperazone, latamoxef and cefodizime. At the same time the concentrations of these drugs in the blood and lungs of infected mice were also determined. The infecting organism used in these studies was *Klebsiella pneumoniae* DT-S, a strain which caused acute pulmonary infection in mice[7~10].

Materials and Methods

Antibiotics

Cefpirome (HR 810, sulfate), cefotaxime and cefodizime (HR 221) were prepared by Hoechst AG, Frankfurt, FRG; ceftazidime, cefoperazone and latamoxef were obtained from a commercial source.

Challenge Organism

The infecting organism, *K. pneumoniae* DT-S, was kindly provided by Takeda Chemical Industries, Ltd., Osaka, Japan. The strain was maintained by intrabronchial passage in mice every 14 days and stored at room temp on Mueller-Hinton agar. For the *in vivo* experiments, the strain was transferred to brain heart infusion agar slants and, after incubation at 37°C for 20 hours, the bacteria were cultured in brain heart infusion broth for 16 hours at 37°C. This culture, containing approximately 5×10^8 colony forming units/ml (cfu/ml), was then used for the infection studies.

The minimum inhibitory concentrations (MICs), defined as the lowest concentrations of the antibiotics that suppressed visible growth after 20 hours incubation at 37°C of an inoculum of 5×10^5 cfu/ml *K. pneumoniae* DT-S, were determined in Mueller-Hinton broth (Difco) as previously described[1,2,5].

Production of Pneumonia; Infection Procedure

Experimental pneumonia was produced as previously described by NISHI and TSUCHIYA[7,8]. In brief, up to 120 male NMRI mice, strain NMKf Hoe SPF 71, were placed in an exposure chamber. Four ml of the *Klebsiella* suspension were put in a nebulizer (Vaponefrin Pocket Nebulizer, USV Pharmaceutical Co., U.S.A.) and nebulized with compressed air at a pressure of one kg/cm^2 for 40 minutes. Half an hour after infection, up to 2.5×10^2 cfu/g tissue of *K. pneumoniae* DT-S were found in the lungs of the exposed mice.

Bactericidal Effect *In Vitro* (Time-kill Curves)

The bactericidal effect of the cephalosporin antibiotics on *K. pneumoniae* DT-S *in vitro* was assessed by killing curve experiments. 18-hours-cultures of *K. pneumoniae* DT-S were suspended in 1 liter of Mueller-Hinton broth at a density of about 10^5 bacteria per ml and incubated on a shaker for 24 hours at 37°C. After two hours, cephalosporins were added in concentrations of approximately equal to the MIC. Samples were removed after 0, 1, 2, 4, 6 and 24 hours of incubation; they were diluted immediately with Mueller-Hinton broth and plated on drug-free Mueller-Hinton agar to determine the cfu.

Klebsiella pneumoniae Time-Kill Curves *In Vivo*

Mice with experimentally induced pneumonia were injected with a 50 mg/kg dose of the test antibiotics 18 hours after infection. They were killed prior to and at intervals between 1 and 48 hours after the administration of antibiotic (ten animals/time point for each group). 0.1 ml of blood was taken from the inferior *vena cava*, plated on Mueller-Hinton agar and incubated for colony counts. The lungs were excised, weighed, diluted in nine times the weight of 10 mM phosphate buffered saline (PBS), homogenized, serially diluted and plated. The cfu were counted after 24 hours incubation at 37°C. Analysis of variance was used for statistical comparisons.

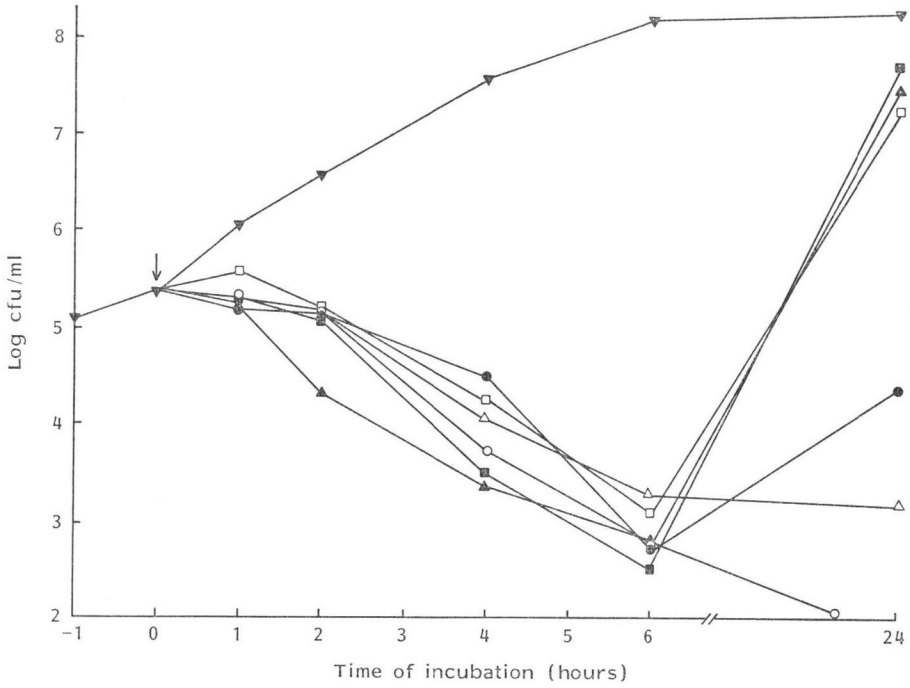
Treatment of Pulmonary Infections

Mice were infected by the aerosol method and the pneumonia was allowed to become well established before treatment was carried out. The antibiotics were injected subcutaneously in serial two-fold dose concentrations as follows; once 18 hours or 28 hours after infection, twice 21 and 28 hours after infection or three times, 18, 19 and 20 hours after infection. The volume administered was 1 ml/mouse. Ten control mice were left untreated. The number of dead mice was recorded daily. On the tenth day after infection, the surviving animals were sacrificed and the lungs removed for the determination of bacterial recovery. The lungs were homogenized with nine times the weight of 10 mM PBS in an Ultra Turrax homogenizer (Janke & Kunkel KG, FRG). Ten-fold serial dilutions of the homogenate in PBS were prepared, and 0.1 ml of the samples was plated on Mueller-Hinton agar plates (Difco). The agar plates were incubated at 37°C for 20 hours and the cfu/g tissue counted. The median effective dose (ED_{50}, mg/kg/dose), *i.e.* the amount of antibiotic required for the survival of 50% of the animals, and the median clearance dose (CD_{50}, mg/kg/dose), *i.e.* the amount of antibiotic required for eradication of *K. pneumoniae* from the lungs of 50% of the infected animals, were calculated by probit analysis.

Fig. 1. Comparative bactericidal activity of cefpirome, ceftazidime, cefotaxime, cefoperazone and latamoxef against *Klebsiella pneumoniae* DT-S *in vitro*.

Each agent was present at once the MIC: Cefpirome, 0.03 μg/ml; ceftazidime, 0.06 μg/ml; cefotaxime, 0.015 μg/ml; cefoperazone, 0.06 μg/ml and latamoxef, 0.25 μg/lm. Arrow, addition of the drugs.

▼ Control, ○ cefpirome, □ ceftazidime, ■ cefotaxime, ▲ cefoperazone, ● latamoxef, △ cefodizime.



Bioassay

The levels of the cephalosporins in murine blood and lung tissue at various intervals after a single subcutaneous injection of 50 mg/kg were determined by agar diffusion technique[6,10,11].

Results

Bactericidal Activity *In Vitro*

A comparison of the bactericidal activity of cefpirome and other cephalosporin antibiotics against *K. pneumoniae* DT-S is shown in Fig. 1. Each drug was present in concentrations approximate to the MIC. In the first six hours of incubation, cefpirome and cefodizime at 0.03 μg/ml were as effective as the other cephalosporins at concentrations of: 0.015 μg/ml (cefotaxime), 0.06 μg/ml (ceftazidime and cefoperazone) and 0.25 μg/ml (latamoxef). But then, a rapid regrowth of *K. pneumoniae* DT-S was observed with four out of five reference compounds, which was not true for cefpirome and cefodizime. Only few bacteria were found after cefodizime incubation, but no viable counts could be detected after 24 hours of cefpirome incubation.

Bactericidal Activity in Pneumonic Mice

In the clearance studies, single sc doses of the cephalosporins (50 mg/kg) were administered 18 hours after challenge when approximately 9×10^5 cfu/g of *K. pneumoniae* DT-S in the lungs of the mice were present. During the first eight hours after medication, cefpirome, cefotaxime, ceftazidime and cefo-

Table 1. Comparative bactericidal effects of cefpirome and reference compounds against *Klebsiella pneumoniae* DT-S in the lungs of pneumonic mice after a single subcutaneous dose of 50 mg/kg, 18 hours after challenge.

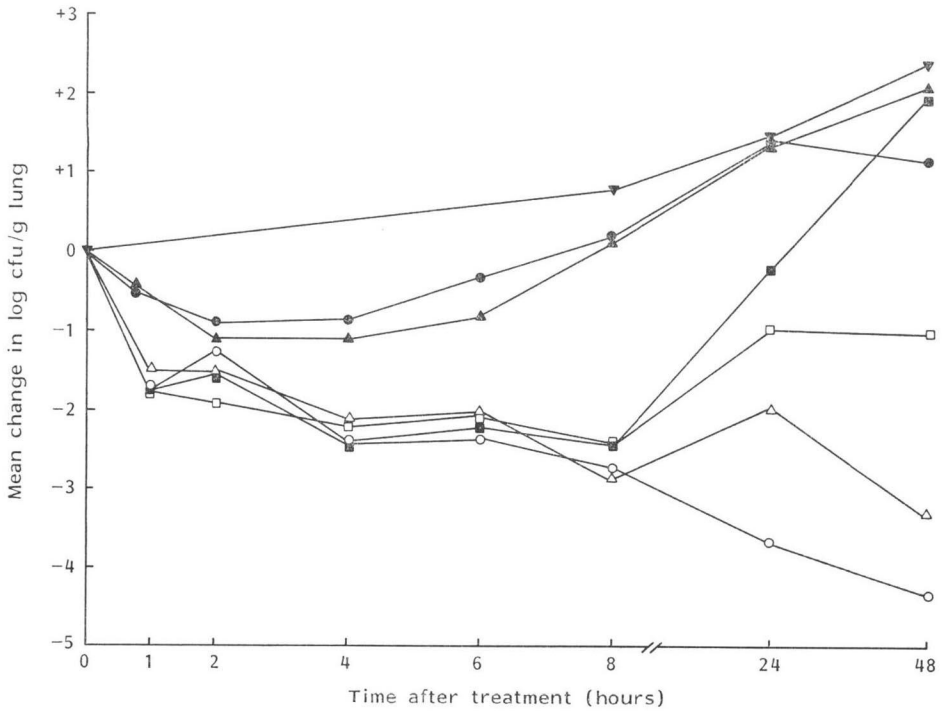
Compound	Mean change in log cfu/g in the lungs at the following times after medication						
	1 h**	2 h**	4 h**	6 h**	8 h**	24 h**	48 h**
None	N.D.	N.D.	N.D.	N.D.	+0.8	+1.5	+2.5
Cefpirome	−1.7	−1.3	−2.4	−2.3	−2.7	−3.7	−4.4
Ceftazidime	−1.8	−1.9	−2.2	−2.1	−2.4	−0.9*	−1.0*
Cefotaxime	−1.7	−1.5	−2.4	−2.2	−2.4	−0.2*	+2.0*
Cefoperazone	−0.4	−1.1	−1.1*	−0.6*	+0.1*	+1.4*	+2.1*
Latamoxef	−0.5	−0.9	−0.9*	−0.3*	+0.2*	+1.3*	+1.2*
Cefodizime	−1.5	−1.5	−2.1	−2.0	−2.9	−1.9	−3.3

N.D.: Not determined.
Initial bacterial titer (18 hours after exposure) was 5.82 log cfu/g lung.
* Values are significantly different from those of cefpirome ($P<0.05$).
** Hours after treatment.

Fig. 2. Comparative bactericidal activity of cefpirome and other cephalosporins against *K. pneumoniae* DT-S *in vivo*: Viable bacteria from the lungs of pneumonic mice after a single subcutaneous injection of 50 mg/kg of the drugs at 18 hours after aerosol exposure.

Each value represents the mean of ten animals. The mean initial bacterial titer in the lungs of untreated mice at 18 hours after infection was 5.8 log cfu/g.

▼ Control, ○ cefpirome, □ ceftazidime, ▩ cefotaxime, ▲ cefoperazone, ● latamoxef, △ cefodizime.



dizime all displayed similar killing capacity and caused a mean reduction in the viable cell count in the lungs of 2.4~2.7 log cfu/g (Table 1 and Fig. 2). With cefpirome and, to a smaller extent, also with cefodizime, a further reduction in challenge organisms by up to 1/10,000 of the pretreatment level was

Table 2. Therapeutic activity of cefpirome and other cephalosporins on experimentally induced *K. pneumoniae* respiratory tract infections in mice: Median effective doses (ED_{50}s) after various treatment regimens.

Compound	ED_{50} (mg/kg/dose)			
	18 h*	28 h*	21+28 h*	18+19+20 h*
Cefpirome	15.4	51.0	3.2	2.0
Ceftazidime	28.5	189.1	11.4	5.5
Cefotaxime	108.6	217.0	20.6	19.8
Cefoperazone	>1,000.0	>1,000.0	199.5	192.5
Latamoxef	286.8	377.0	41.8	27.1
Cefodizime	3.2	59.1	2.0	1.1

* Hours of treatment after infection.

Table 3. Therapeutic acitvity of cefpirome and reference compounds on experimentally induced *K. pneumoniae* respiratory tract infections in mice: Median clearance doses (CD_{50}s) after various treatment regimen.

Compound	CD_{50} (mg/kg/dose)			
	18 h*	28 h*	21+28 h*	18+19+20 h*
Cefpirome	18.6	191.5	6.8	5.6
Ceftazidime	50.9	1,013.5	25.0	22.9
Cefotaxime	193.7	594.5	56.7	43.6
Cefoperazone	>1,000.0	>1,000.0	709.5	636.5
Latamoxef	620.0	>1,000.0	117.7	51.7
Cefodizime	25.0	393.9	10.3	4.9

* Hours of treatment after infection.

observed and in eight out of ten and five out of ten animals, respectively, no bacteria could be found in the lungs at 48 hours. Bacteremia did not occur in any of the test animals in each group. With ceftazidime and, in particular, with cefotaxime, a rapid regrowth could be observed at 24 and 48 hours (Fig. 2), and bacteria were eradicated from the lungs of only two of the ten mice in the ceftazidime group and from none in the cefotaxime group. Moreover bacterial blood titers at 48 hours were detected in eight out of ten mice in each group. Despite their good antibacterial activity *in vitro* and despite the high latamoxef-levels in the infected lungs (Table 4), latamoxef as well as cefoperazone, exhibited only a slight bactericidal effect *in vivo*, and the bacterial growth rate in treated mice was similar to that seen in the controls. At 48 hours after cefoperazone or latamoxef treatment, high bacterial counts were determined not only in the lungs but also in the blood of nearly all the mice.

Effects of Cephalosporin Treatment

Tables 2 and 3 compare the chemotherapeutic activity and clearance efficacy of cefpirome and five other cephalosporin derivatives against experimental *Klebsiella* respiratory tract infections in mice. In all treatment regimens used, cefpirome and cefodizime were the most effective compounds tested, followed by ceftazidime and cefotaxime. Latamoxef was less active, whereas with cefoperazone successful therapy was achieved only when the compound was given two or three times in high dosages. Treatment of pneumonic mice and the eradication of the challenge organism from the lungs was substantially more difficult when started late after aerosol exposure. Thus, at 28 hours, it was necessary to increase the cephalosporin amount by between 2 and 18 times in order to obtain a similar effect to that achieved by therapy at 18 hours. Lower dosages had to be given when treatment regimens

Table 4. Concentrations of various cephalosporins in the blood and lung tissue of pneumonic mice.

Antibiotic	Sample	Limit of detection (μg/ml)	Concentration (μg/ml or μg/g)				
			1 h*	2 h*	4 h*	6 h*	8 h*
Cefpirome	Blood	0.1	14.6±4.1	3.2±0.7	N.D.	N.D.	N.D.
	Lungs	0.05	10.4±3.1	2.6±0.9	0.3±0.2	N.D.	N.D.
Ceftazidime	Blood	1.6	17.7±4.9	1.8±0.9	N.D.	N.D.	N.D.
	Lungs	0.2	7.6±1.7	N.D.	N.D.	N.D.	N.D.
Cefotaxime	Blood	0.8	20.3±5.9	2.2±0.8	N.D.	N.D.	N.D.
	Lungs	0.1	3.5±1.3	0.1±0.1	N.D.	N.D.	N.D.
Cefoperazone	Blood	3.1	6.4±1.4	N.D.	N.D.	N.D.	N.D.
	Lungs	0.8	N.D.	N.D.	N.D.	N.D.	N.D.
Latamoxef	Blood	1.6	25.5±3.8	5.8±0.8	N.D.	N.D.	N.D.
	Lungs	0.4	14.2±5.8	3.2±1.3	N.D.	N.D.	N.D.
Cefodizime	Blood	0.8	81.5±21.5	29.8±4.8	8.6±2.1	2.8±0.8	0.8±0.9
	Lungs	0.4	46.7±15.3	17.8±7.4	5.1±1.6	1.8±3.5	N.D.

N.D.: Not detectable, *i.e.*, no concentrations above the limits of detection were observed.
* Hours after single dose of 50 mg/kg.

involving two or three-fold administration were chosen (Tables 2 and 3).

Concentrations in the Blood and Lung Tissue of Pneumonic Mice

Table 4 demonstrates the antibiotic levels in the blood and lungs of mice injected subcutaneously with 50 mg/kg of the various cephalosporins. Extremely high and prolonged levels in the blood and lung tissue of pneumonic animals, up to 81.5 μg/ml and 46.7 μg/ml, respectively, were found after cefodizime injection. Higher blood levels could be observed at 1 hour with cefotaxime and latamoxef (20.3 and 25.5 μg/ml, respectively) than with cefpirome (14.6 μg/ml). Only low concentrations (6.4 μg/ml) were found in animals treated with cefoperazone. Comparatively high levels of cefpirome and latamoxef were detected in the lungs of pneumonic mice (10.4 and 14.6 μg/g, respectively), whereas ceftazidime and cefotaxime delivered lower concentrations (7.6 and 3.5 μg/g, respectively). The lung level of cefoperazone was below the limit of detection (0.8 μg/ml) before 1 hour after injection.

Discussion

Respiratory tract infections caused by Gram-negative bacteria remain difficult diseases to treat. As a result of their abundant appearance in hospitalized patients in intensive care units and in patients with malignancies, they are characterized by long lasting morbidity and high mortality rates[12~15]. For this reason, there is pressing need for novel antibacterial drugs which combine high activity against the infecting organisms with good diffusibility to the sites of infection in the tissue. Recent investigations using cefpirome in our laboratories demonstrated its broad-spectrum activity, which includes Gram-positive and Gram-negative bacteria, its good pharmacokinetics and its high efficacy against systemic and localized infections in experimental animals[1,2,5,6]. In the trials presented in this paper we assessed the ability of cefpirome and five other cephalosporin antibiotics to treat and eradicate experimental pneumonia caused by *K. pneumoniae* DT-S in mice.

In spite of the challenge organism being equally sensitive in MIC studies to cefpirome and four of the reference compounds and despite similar pharmacokinetic patterns in the blood of mice, the data show cefpirome superiority over comparable cephalosporins due to comparatively good distribution and, in particular, a more marked bactericidal activity against *K. pneumoniae* DT-S. Primarily, regrowth of bacteria in the lungs of the infected animals and the exacerbation of infection were prevented by cefpirome but not by cefotaxime, ceftazidime, cefoperazone or latamoxef medication. Therefore,

distinctly lower amounts of cefpirome were required for successful therapy than of the references. Cefodizime showed similar activity *in vitro* to cefpirome against Enterobacteriaceae, but differed substantially in its pharmacokinetic features from cefpirome and the other cephalosporins tested[9,10,11]. This and its comparatively good bactericidal activity proved to be one of the reasons for its high efficacy also in this model.

Although caution must be excised in the extrapolation of findings obtained in animal models to the clinical situation, the results presented suggest that cefpirome may be useful in treating acute forms of pulmonary infection caused by Gram-negative rods.

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