CEFODIZIME, AN AMINOTHIAZOLYL CEPHALOSPORIN

III. THERAPEUTIC ACTIVITY AGAINST EXPERIMENTALLY INDUCED PNEUMONIA IN MICE

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The activity of the aminothiazolyliminomethoxy cephalosporin cefodizime (HR 221) was compared to that of cefotaxime, cefuroxime and cefazolin in experimental pneumonia caused by *Klebsiella pneumoniae* DT-S in mice. Cefodizime exhibited high and long-acting levels in the blood and lung homogenates of infected mice; the blood and tissue concentrations obtained with the other cephalosporins tested were low by comparison. In the treatment of experimental *Klebsiella* pneumonia, cefodizime was superior to cefotaxime and cefuroxime. Counts of the number of viable bacteria present in the infected tissue showed that cefodizime exerted a more marked bactericidal effect than cefotaxime or cefuroxime. Hardly any therapeutic activity was seen with cefazolin.

Gram-negative bacillary pneumonias represent an increasing problem in hospitalized patients, particularly in chronically ill patients in intensive care units and patients with malignancies. Pneumonias caused by such bacteria are extremely difficult to control and are characterized by high mortality rates. One of the most common infecting agents is *Klebsiella pneumoniae*, which accounts for 25% of Gram-negative nosocomial pneumonias[1~5]. NISHI and TSUCHIYA previously described the production of lobar pneumonia in mice using the aerosol method and *K. pneumoniae* DT-S as the infecting organism[6~8]. Although this model reproduces only the acute phase of the disease in humans, and the majority of the experimental animals die by day-three after infection, there are some economic, practical and statistical advantages of using a rodent model to study the infectious process or to evaluate the antibacterial activity of newer compounds.

This paper reports on the therapeutic efficacy of the long-acting aminothiazolyl cephalosporin cefodizime (HR 221) in experimentally induced lung infections in mice as compared with that of cefotaxime, cefuroxime and cefazolin. The challenge organism used in these studies was *K. pneumoniae* DT-S, a bacterial strain with a high virulence towards mice[6]. The concentrations of the test antibiotics in the blood and lungs of infected mice were also determined.

Materials and Methods

Antibiotics

Cefodizime (HR 221) and cefotaxime (Claforan) were prepared by Hoechst AG, Frankfurt, FRG. Cefuroxime (Zinacef, Hoechst/Glaxo, Frankfurt, FRG) and cefazolin (Gramaxin, Boehringer Mannheim GmbH, Mannheim, FRG) were obtained from a commercial source.

Antimicrobial Susceptibility Tests

The minimum inhibitory concentrations (MICs), defined as the lowest concentrations of the antibiotics that suppressed visible growth after 20-hour 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[9].

Challenge Organism

The infecting organism, *K. pneumoniae* DT-S, was kindly provided by Takeda Chemical Industries, Ltd., Osaka, Japan. This strain was maintained by intrabronchial passage in mice every 14 days and was stored at room temperature on Trypticase soy 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 units cfu per ml, was then used for the infection studies.

Measurement of Antibiotic Concentrations in Blood and Lung Tissue

Pneumonic mice were dosed subcutaneously with 50 mg/kg of the four cephalosporins tested. Blood samples were removed from a cut on the tip of the tail by means of 10 μl capillary tubes (Wiretrol, mfr.: Drummond, Broomall, USA). Mice were killed by exsanguination and lungs were removed, homogenized with four times the weight of phosphate buffer (pH 6.0) and assayed for their antibiotic concentrations. The levels of the cephalosporins in the blood and lung samples were determined microbiologically by the agar diffusion technique as previously described[10]. *Streptococcus pyogenes* A 77 was used as the indicator strain. The standard solutions and diluents of each of the compounds tested were prepared with murine blood for blood assays and with homogenate of untreated lung tissue (tissue/buffer ratio 1: 5) for determining the concentrations in lung tissue.

Production and Treatment of Experimentally Induced Septicaemia

Protection tests were carried out as previously described[11]. The test animals were each infected intraperitoneally with 0.3 ml of a bacterial suspension containing 2×10^4 cfu, *i.e.* approximately 10 times the median lethal dose (LD_{50}), of *K. pneumoniae* DT-S. The infected mice of the untreated control groups died within 24 hours of infection. Ten mice were used for each of the serial two-fold dose concentrations of the test cephalosporins. The treatment was subcutaneous-immediately and 4 hours after infection.

Production of Pneumonia, Infection Procedure

Experimental pneumonia was produced as previously described by NISHI and TSUCHIYA[6~8]. In brief, up to 120 male NMRI mice, strain NMKf Hoe SPF 71, were placed in an exposure chamber. 4 ml of the *Klebsiella* suspension were put in a nebulizer (Vaponefrin Pocket Nebulizer, USV Pharmaceutical Co., USA) and were nebulized with compressed air at a pressure of 1 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.

Treatment of *K. pneumoniae* Lung Infections with Various Antibiotic Regimens

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 or twice, 21 and 28 hours after infection. The volume administered was 1 ml/mouse. 10 control mice were left untreated.

The number of dead mice was recorded daily. On day 10 after infection, the surviving animals were sacrificed and the lungs removed for determination of bacterial recovery. The lungs were homogenized with nine times the weight of 10 mM phosphate buffered saline (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 were counted. The median effective dose (ED_{50}, mg/kg), *i.e.* the amount of antibiotic required for survival of 50% of the animals, and the median clearance dose (CD_{50}, mg/kg), *i.e.* the antibiotic amount required for eradication of *K. pneumoniae* from the lungs of 50% of the infected animals, were calculated by probit analysis.

K. pneumoniae Time-kill Curves *In Vivo*

Mice with experimentally induced pneumonia were injected with 50 mg/kg of the test antibiotics

18 hours after infection. Groups of ten mice were killed prior to and at intervals between 1 and 48 hours after antibiotic administration. 0.1 ml of blood was taken from the inferior vena cava, plated on meat extract agar and incubated for colony counts. The lungs were excised, weighed, diluted in nine times the weight of PBS, homogenized, serially diluted and plated. The cfu were counted after 24-hour incubation of 37°C. The detection limits for blood and lungs were 10^1 and 10^2 cfu/g respectively.

Table 1. Comparison of the activities of cefodizime and other cephalosporins against *K. pneumoniae* DT-S *in vitro* and in experimentally induced *Klebsiella* septicaemia in mice.

Antibiotic	MIC (μg/ml)	ED_{50} (mg/kg)
Cefodizime	0.03	0.9
Cefotaxime	0.004	0.8
Cefuroxime	0.5	47.8
Cefazolin	1.0	136.1

Results

In Vitro Activity and Effect on Experimental Septicaemia in Mice

The MICs for *K. pneumoniae* DT-S were 0.03 μg/ml (cefodizime), 0.004 μg/ml (cefotaxime), 0.5 μg/ml (cefuroxime) and 1.0 μg/ml (cefazolin) (Table 1).

In the protection test, cefodizime and cefotaxime both showed the same efficacy and were 60~170 times more effective than cefuroxime and cefazolin (Table 1).

Antibiotic Levels in the Blood and Lungs

Table 2 shows the antibiotic concentrations in the blood and lungs of mice injected subcutaneously with 50 mg/kg of the various cephalosporins. Cefodizime showed extremely high and prolonged levels in the blood and lung tissue of infected animals. Concentrations many times the MIC of the compound for the challenge organism were still observed even 6 and 8 hours after dosing. In contrast, blood and lung levels of cefotaxime, cefuroxime and cefazolin were only measurable up to 1 or 2 hours after injection.

Experimental Pneumonia

Evaluation of Infection in Control Mice

Mice were killed at various intervals. The lungs of all the test animals were infected but the blood cultures remained negative for approximately two days after exposure (Fig. 1). Whereas, 42 hours

Table 2. Concentrations of cefodizime and reference compounds in the blood and lung tissue of mice with experimentally induced *Klebsiella* lung infections.
Treatment: 50 mg/kg once, 18 hours after infection; each value is the mean of ten animals.

Antibiotic	Sample	Limit of detection (μg/ml)	Concentration (μg/ml), hour after medication				
			1	2	4	6	8
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	ND
Cefotaxime	Blood	0.8	37.7±11.4	2.2±0.8	ND	ND	ND
	Lungs	0.1	3.5± 1.3	0.1±0.1	ND	ND	ND
Cefuroxime	Blood	0.4	26.4±10.0	3.7±1.8	ND	ND	ND
	Lungs	0.05	10.9± 1.4	1.4±0.6	ND	ND	ND
Cefazolin	Blood	1.6	12.9± 4.1	ND	ND	ND	ND
	Lungs	0.4	9.2± 3.6	ND	ND	ND	ND

ND: Not detectable.

Fig. 1. Viable *K. pneumoniae* DT-S from the lung tissue and blood of untreated mice after aerosol challenge.

Each value represents the mean of ten mice.

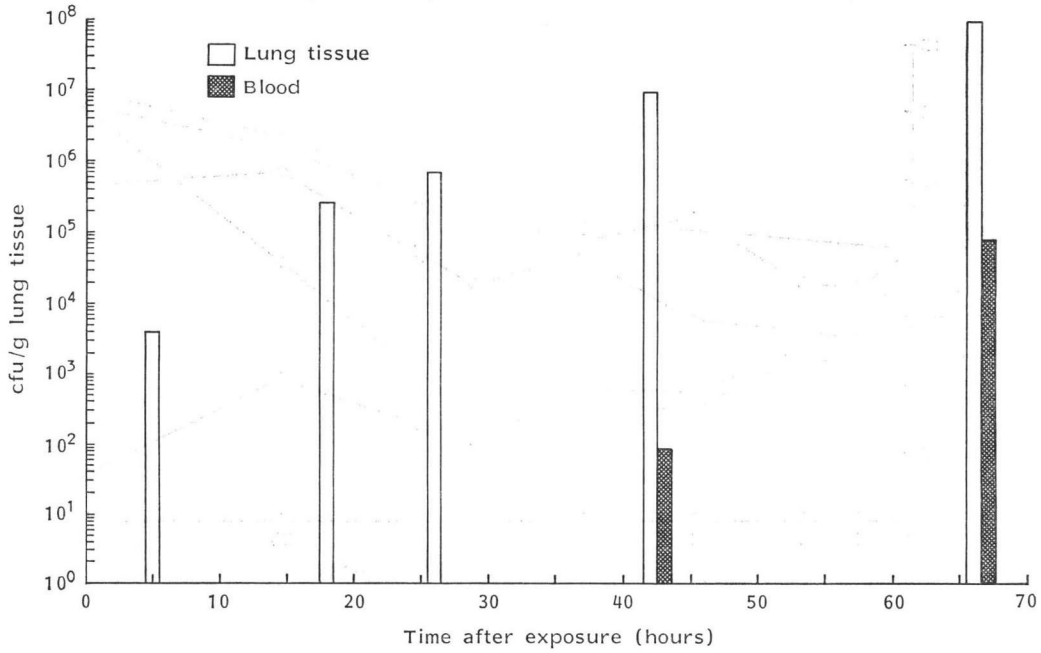


Table 3. Comparison of the effects of cefodizime and reference compounds on experimentally induced *K. pneumoniae* lung infections in mice.

Antibiotic	Treatment: once, 18 hours after infection		Treatment: twice, 21 and 28 hours after infection	
	ED_{50}	CD_{50}	ED_{50}	CD_{50}
Cefodizime	3.2	25.0	2.0	10.3
Cefotaxime	63.0	173.9	22.4	28.7
Cefuroxime	1,646.0	>2,000.0	395.9	1,013.5
Cefazolin	>2,000.0	>2,000.0	>2,000.0	>2,000.0

Mean effective dose (ED_{50}) and mean clearance dose (CD_{50}) in mg/kg.

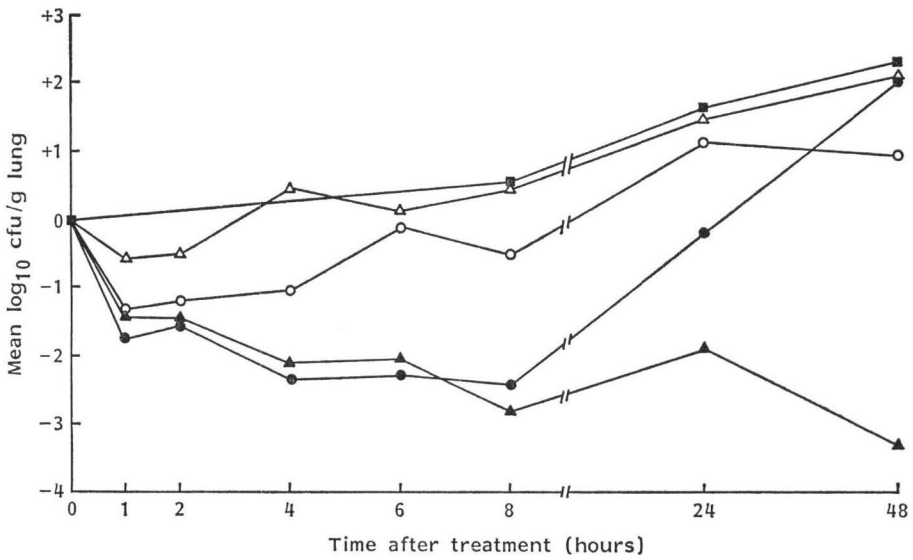
after challenge, up to 10^7 cfu/g tissue of *K. pneumoniae* DT-S were detected in the lungs, the mean number of cfu found in the blood of mice was only 10^2. Generalization of infection occurred by day three after aerosol exposure when bacterial counts were also found in the blood of the control animals. All the untreated mice died within 4 days of exposure.

Effect of Cephalosporin Treatment

Depending on when the treatment was started, great differences in the outcome of therapy were observed.

Where medication was given 18 hours after infection, a comparatively low ED_{50} and CD_{50} of cefodizime (3.2 and 25.0 mg/kg, respectively) were obtained. Cefotaxime, with an ED_{50} of 63.0 and CD_{50} of 173.9 mg/kg, was significantly less active, and cefuroxime and cefazolin were active only at markedly

Fig. 2. Bacterial numbers in the lung tissue of pneumonic mice after a single subcutaneous administration of 50 mg/kg of cefodizime, cefotaxime, cefuroxime or cefazolin at 18 hours after aerosol challenge.
Each value represents the mean of ten mice.
■ Control, ▲ cefodizime, ● cefotaxime, ○ cefuroxime, △ cefazolin.



higher concentrations or proved to be inactive (Table 3).

Twenty-eight hours after aerosol exposure, when up to 2×10^6 cfu/g lung homogenate were detected, medication of pneumonia was substantially more difficult and much higher amounts of cephalosporins were required for successful therapy. For example, it was necessary to increase the cefodizime dosage by at least 35 times to obtain an effect similar to that achieved by medication at 18 hours (not shown).

When the agents were administered twice, 21 and 28 hours after challenge, the therapeutic effect observed was more marked than that obtained with single-dose therapy (Table 3). The activity of cefodizime was again considerably superior to that of cefotaxime and cefuroxime. Cefazolin proved inactive even at a total dose of 2,000 mg/kg. Elimination of the challenge organism from the lungs of 50% of infected mice required 10.3 mg/kg of cefodizime. The corresponding doses of cefotaxime and cefuroxime were 28.7 and 1,013 mg/kg respectively.

Evaluation of the Bactericidal Effect *In Vivo*

Fig. 2 shows the viable counts of *K. pneumoniae* DT-S in the lungs of infected mice after subcutaneous dosing with the various cephalosporins tested. The antibiotics were administered as a single dose of 50 mg/kg 18 hours after exposure, a time when up to 10^6 cfu/g could be detected in the lungs of pneumonic mice. In mice treated with cefodizime, a marked bactericidal effect occurred and the number of bacteria in the lungs fell continuously during the first 4 hours after medication. The viable counts decreased steadily to approximately 1/1,000 of the pretreatment level. The compound sterilized the lungs in 50% of the mice within 48 hours. No positive blood cultures could be detected in any of the animals treated with this drug. Cefotaxime also produced a pronounced antibacterial effect during the first 8 hours, but then, in contrast to cefodizime, the bacteria multiplied rapidly to give a count at

48 hours only slightly below that in the untreated controls. Then, eight out of ten animals showed bacterial counts (up to 10^7 cfu/ml) in the blood. With cefuroxime, only a slight bactericidal effect was observed during the first hour after medication. The bacterial growth rate paralleled that seen in the control animals. Bacteraemia occurred in some mice 24 hours after cefuroxime treatment, *i.e.* 42 hours after exposure. Hardly any therapeutic effects were observed with cefazolin.

Discussion

Cefodizime, cefotaxime, cefuroxime and cefazolin were evaluated for their therapeutic efficacy against experimentally induced *K. pneumoniae* respiratory tract infection in mice. The pathological characteristics of this experimental murine pneumonia were the same as those previously described by NISHI and TSUCHIYA[6~8]. The model reproduces closely the acute pattern of *Klebsiella* sp. pneumonia in humans including massive lobar consolidation and abscess formation with massive cavitation. Bacteraemia occurred as a complication of the fatal infection[4,12].

Despite the higher sensitivity of the infecting organism to cefotaxime *in vitro* and a similar activity in septicaemia tests, the therapeutic effect of cefotaxime on local infections was considerably less than that of cefodizime. From the results of our studies, it would appear that the difference between the therapeutic activities of these two aminothiazolyl cephalosporins in experimental pneumonia is related to the pharmacokinetic behavior of the drugs in mice. The ability of cefodizime to diffuse in tissues and to establish high and persistent levels at the site of infection is more marked than that of cefotaxime.

Cefuroxime and cefazolin showed neither favorable *in vitro* activity nor adequate concentrations in the blood and tissue of infected mice. Both drugs exhibited only low efficacy in this model or proved to be inactive.

In conclusion, these experiments have shown that the *K. pneumoniae* model is not only suitable for studying the effects of antibiotics *in vivo* but can also be used to discriminate between drugs. The long-acting aminothiazolyl cephalosporin cefodizime had a prolonged bactericidal activity and was found to be therapeutically considerably more effective than cefotaxime, cefuroxime and cefazolin.

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