

THERAPEUTIC EFFICACY OF β -LACTAM AND AMINOGLYCOSIDE
ANTIBIOTICS ON EXPERIMENTAL PNEUMONIA CAUSED
BY *KLEBSIELLA PNEUMONIAE* B-54 IN DIABETIC MICE

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The therapeutic efficacy of six β -lactam and aminoglycoside antibiotics were compared in diabetic mice with experimentally induced *Klebsiella pneumoniae* pneumonia. β -Lactams caused a reduction in the numbers of bacteria, with clearance of bacteria from the lungs of diabetic and normal mice. The effect in diabetic mice, however, was very poor. In contrast thereto, no remarkable difference was seen between diabetic and normal mice when treated with aminoglycosides. The concentration of the test antibiotics to the lungs in diabetic mice was lower than in normal mice. The aminoglycosides were more effective than the β -lactams. These data suggest that in treating acute and more chronic forms of pulmonary infection caused by *K. pneumoniae* in diabetic mice aminoglycoside antibiotics are particularly valuable, whereas β -lactams must be given in large quantities using multiple administrations.

It is a well known clinical axiom that patients with diabetes mellitus have an increased susceptibility to various bacterial and fungal infections. Infection remains one of the most serious complications of diabetes mellitus and the three most common sites of infection are the lungs, the skin and the urinary tract. Furthermore, experimental infections in animal with chemically-induced diabetes have shown increased susceptibility to various infection¹⁻⁶⁾. On the contrary some other studies have shown no increased susceptibility to infection^{7,8)}. The diabetic patient is more difficult to treat because of depression of the host defense functions against infection. Not much work has been reported for the treatment of bacterial infection in diabetic animals.

In this report, we have studied the response of alloxan-induced diabetic mice with pulmonary infection to selected antibiotics.

Materials and Methods

Bacterial Strain

Klebsiella pneumoniae B-54 strain, supplied by Prof. K. MATSUMOTO, Institute for Tropical Medicine, Nagasaki University, Nagasaki, Japan, was used in this study. This strain was maintained on nutrient agar slants (Nissui, Tokyo) at room temp.

Antimicrobial Agents

The antimicrobial agents were supplied by the following organizations: cefmenoxime, Takeda Chemical Industries, Ltd., Osaka; ceftizoxime, Fujisawa Pharmaceutical Co., Ltd., Osaka; cefotaxime, Hoechst Japan Ltd, Tokyo; latamoxef and gentamicin, Shionogi & Co., Ltd., Osaka; and dibekacin, Meiji Seika Kaisha, Ltd., Tokyo. Solutions of antimicrobial agents were prepared freshly each day.

Animals

Std-*ddY* male mice weighing 17 to 18 g were obtained from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, and fed water and standard laboratory food.

Method of Producing Diabetes

Mice were fasted overnight and given one intraperitoneal injection of 250 mg/kg of alloxan monohydrate (Kanto Chemical Co. Ltd., Tokyo) dissolved in sterile physiological saline immediately before use. Fasting was continued for another 4 hours after which the animals were returned to their ordinary pellet diet. Six days after alloxan injection, blood was collected from the retroorbital plexus after withholding food for 18 hours. Blood sugar levels of treated and control animals were estimated by the method of WERNER *et al.*⁹⁾. Only those alloxan-treated mice with blood glucose ranging from 140 to 320 mg/dl were used in the experiments.

In Vitro Antimicrobial Activity

The minimum inhibitory concentrations (MICs) of antibiotics against *K. pneumoniae* B-54 were determined by the agar dilution method in sensitive test agar (Eiken, Tokyo). The MIC was defined as the lowest concentration of antibiotic inhibiting growth after overnight incubation at 37°C. The bactericidal activity of β -lactam and aminoglycoside antibiotics *in vitro* was determined in heart infusion broth (Nissui, Tokyo). In brief, stock culture was grown for 18 hours at 37°C in heart infusion broth and subsequently diluted with the same medium to a cell density of approximately 10^7 cfu/ml. Aliquots of 9.0 ml of this inoculated medium were transferred to flasks. The flasks were placed in a water bath at 37°C and 1.0 ml of medium containing 10-fold the desired initial concentration of antibiotics were added. The flasks were incubated at 37°C, 0.5 ml aliquots were removed at intervals, diluted in saline, and plated for viable cell count determination. Colonies were counted after 24 hours of incubation at 37°C and the number of viable bacteria in the samples was estimated.

Bactericidal Activity *In Vivo*

Experimental pneumonia was induced by the aerosol method as described by NISHI *et al.*¹⁰⁾ with slight modification. For this purpose, *K. pneumoniae* B-54 was grown on nutrient agar for 18 hours at 37°C and cells harvested from the slant were suspended in nutrient broth (Nissui) to give a final cell density of *ca.* 10^9 cfu/ml. The test antibiotics were administered subcutaneously at 15 hours after infection of the mice. At intervals, animals were bled to death and their lungs were removed aseptically. The lungs were homogenized in saline, and dilutions were plated for colony counts.

Concentration of the Antibiotics in the Lungs

The test antibiotics were administered by subcutaneous routes in single doses of 125 mg/kg (β -lactam antibiotics) and 100 mg/kg (aminoglycosides) each to four mice infected with *K. pneumoniae*. Concentrations in the lungs were determined microbiologically by a disk plate assay method with *Proteus mirabilis* ATCC 21100 as test strain for β -lactams and with *Staphylococcus epidermidis* KP-1 for aminoglycosides.

Table 1. MICs of β -lactams and aminoglycosides against *Klebsiella pneumoniae* B-54.

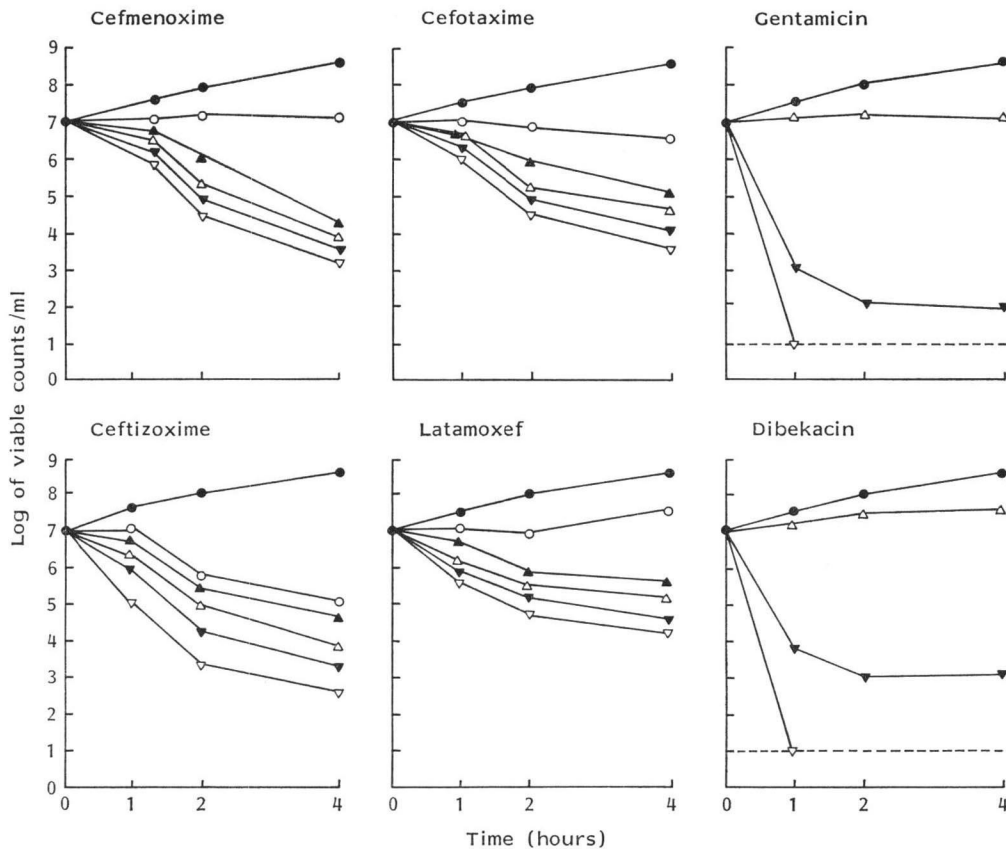
Antibiotic	MIC (μ g/ml) at inoculum	
	10^6 cfu	10^8 cfu
Cefmenoxime	0.025	0.05
Ceftizoxime	≤ 0.006	0.006
Cefotaxime	0.025	0.05
Latamoxef	0.05	0.1
Gentamicin	0.39	0.39
Dibekacin	0.39	0.78

Results

In Vitro Antimicrobial Activity

The MICs of β -lactams and aminoglycosides against *K. pneumoniae* are shown in Table 1. The MICs were 0.025 μ g/ml for cefmenoxime and cefotaxime, ≤ 0.006 μ g/ml for ceftizoxime, 0.05 μ g/ml for latamoxef, and 0.39 μ g/ml for gentamicin and dibekacin with inoculum sizes of 10^6 cfu/ml. *In vitro* bactericidal activities of the antibiotics shown in Fig. 1. Significant reduction in the number of surviving bacterial cells was observed at 0.04 μ g/ml of cefmenoxime, cefotaxime, and latamoxef and at 0.008 μ g/ml of ceftizoxime with initial cell densities of 10^7 cfu/ml. On the other hand, obvious

Fig. 1. Effect of β -lactams and aminoglycosides on the viability of *Klebsiella pneumoniae* B-54.
 ● Control, ○ 0.008 μ g/ml, ▲ 0.04 μ g/ml, △ 0.2 μ g/ml, ▼ 1 μ g/ml, ▽ 5 μ g/ml.



bactericidal effects of gentamicin and dibekacin were seen at first with 1 μ g/ml, but not with 0.2 μ g/ml. However, the bactericidal effects of aminoglycosides occurred faster than those of the β -lactams, especially during the first hour.

Bactericidal Activity *In Vivo*

Single Administration

The results of protection tests against experimental pneumonia in mice are shown in Table 2. In significant numbers of normal mice β -lactams caused a reduction of bacterial cells, with clearance of the organisms from the lungs at 125 mg/kg or more. In diabetic mice, the reduction by β -lactams of bacterial cells in the lungs, obtained by a dosage of 125 to 250 mg/kg occurred to a much lower extent than in normal mice. Aminoglycosides caused a clearance of bacteria from the lungs already at 25 mg/kg or more and no remarkable difference was seen between diabetic mice and control mice.

Multiple Administration

The efficacies of cefmenoxime and ceftizoxime in multiple administration are shown in Figs. 2 and 3. The test antibiotics were administered once, twice, and three times with a two-hour interval between the doses. On the basis of the times of administration, β -lactams caused reduction in the numbers of bacteria in the lungs, with clearance of bacteria from the lungs, and suppression of regrowth in

Table 2. Therapeutic efficacies of β -lactams and aminoglycosides after subcutaneous administration on the number of bacteria in the lung of normal and diabetic mice infected with *Klebsiella pneumoniae* B-54 by the aerosol method.

Drug (mg/kg)	Log of viable counts/lung, hour after administration								
	Normal mice				Diabetic mice				
	0	4	24	48	0	4	24	48	
Cefmenoxime	250	—	2.83	2.00	2.53	—	3.95	5.52	7.00
	125	—	3.36	4.00	4.64	—	4.67	6.59	7.68
	62.5	—	4.07	6.03	8.01	—	5.06	7.11	8.03
Ceftizoxime	250	—	2.87	2.22	2.84	—	3.11	4.09	6.02
	125	—	3.45	3.22	4.10	—	4.02	4.99	7.15
	62.5	—	4.08	5.01	7.23	—	4.98	6.70	7.81
Cefotaxime	250	—	3.00	4.52	6.00	—	4.00	6.45	7.85
	125	—	3.50	4.85	7.01	—	4.50	6.75	8.20
	62.5	—	4.45	6.23	8.00	—	5.56	7.11	8.48
Latamoxef	250	—	2.01	3.03	6.48	—	3.00	6.02	7.48
	125	—	2.45	4.50	7.98	—	3.52	6.99	8.48
	62.5	—	3.52	6.80	8.10	—	4.52	7.30	8.70
Gentamicin	100	—	1.00	1.03	1.48	—	1.40	1.55	2.03
	50	—	2.02	2.48	3.03	—	2.48	2.80	3.20
	25	—	2.88	2.93	5.00	—	3.21	3.75	5.65
Dibekacin	100	—	2.00	2.03	2.65	—	1.85	1.57	1.98
	50	—	2.15	2.59	4.85	—	2.13	2.11	5.60
	25	—	2.98	4.30	6.03	—	2.48	3.30	6.00
Untreated control		5.58	5.68	7.68	8.26	6.04	6.24	8.04	8.50

Each value represents the mean of five mice.

Fig. 2. Therapeutic efficacy of cefmenoxime on number of bacteria in the lung of normal and diabetic mice infected with *Klebsiella pneumoniae* B-54 by the aerosol method.

Each value represents the mean of five mice.
 ○; Normal mice, ●; diabetic mice.

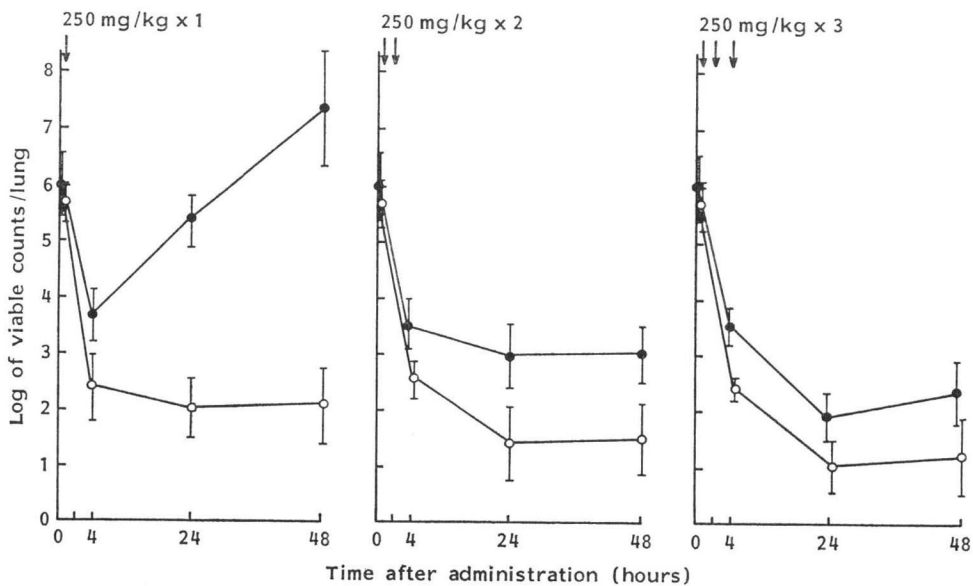


Fig. 3. Therapeutic efficacy of ceftizoxime on number of bacteria in the lung of normal and diabetic mice infected with *Klebsiella pneumoniae* B-54 by the aerosol method.

Each value represents the mean of five mice.
 ○; Normal mice, ●; diabetic mice.

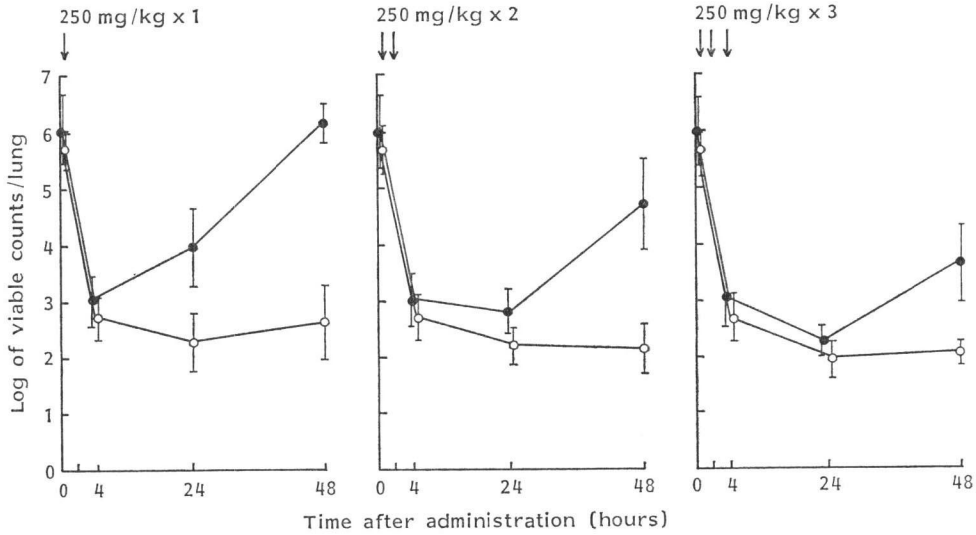
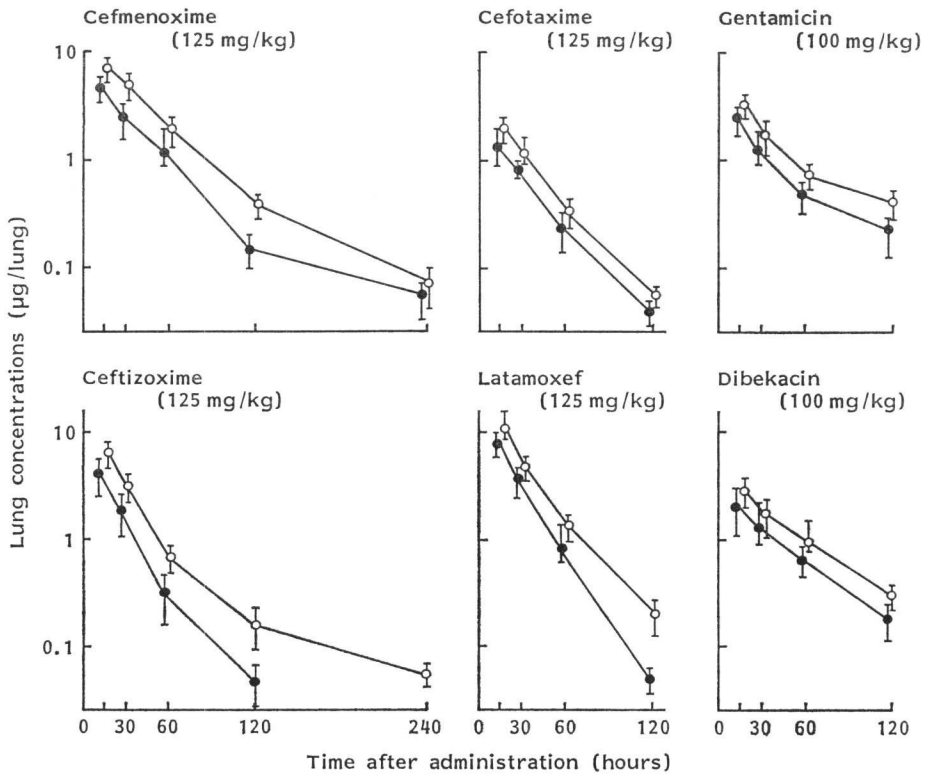


Fig. 4. Lung concentration of several antibiotics after subcutaneous injection to normal and diabetic mice infected with *Klebsiella pneumoniae* B-54 by the aerosol method.

○; Normal mice, ●; diabetic mice.



the lungs. The efficacy of a single administration in normal mice was equivalent to the efficacy of three times administration in diabetic mice.

Lung Concentrations

The mean lung concentrations obtained after 125 mg/kg of β -lactams and 100 mg/kg of aminoglycosides are shown in Fig. 4. A mean peak level was obtained 15 minutes after application and there was an initial rapid distribution phase followed by a gradual and steady decline. The mean peak level in diabetic mice, however, was only 70 to 80% of that of normal mice.

Discussion

Although much work has been done to investigate bacterial and fungal infections in animals with alloxan and streptozotocin induced diabetes mellitus, few reports are available on treatment of bacterial infections. In this paper, the therapeutic efficacy of β -lactams and aminoglycosides in diabetic mice were evaluated. On the basis of the dose-response relationship, β -lactams caused a reduction in the number of bacteria, with clearance of *K. pneumoniae* from the lungs of diabetic and normal mice. A remarkable difference was however seen between the effect in diabetic mice and that in normal mice, elimination of bacteria from the former being very poor. This might be due to the lower distribution of parenteral drugs in the lungs of diabetic mice (Fig. 4). Following treatment of animals with aminoglycosides, there was remarkably no difference in clearance of bacteria from the lungs between normal and diabetic mice (Table 2), though the distribution of these antibiotics to the lungs was shown to be as low as in the case of β -lactams. The obvious advantage of aminoglycosides seems to depend on their faster and stronger bactericidal activity, as this was observed *in vitro* (Fig. 1). Nevertheless, the side effects and toxicity of these compounds must be kept in view. β -Lactam antibiotics are known to cause the development of filamentous cells over a wide range of relatively low concentrations. Since filamentous cells are able to resume cell division and growth after removal of the antibiotics, the poor *in vivo* activity of β -lactams in diabetic mice might be attributed to a combined disadvantage of the low distribution characteristic and low bactericidal activity of the compounds. Some workers¹¹⁻¹³) reported that after treatment with β -lactams, such as sulbenicillin, cefuroxime, and ceftizoxime, the filamentous cells were more susceptible to non-specific host defense functions, such as serum and neutrophils, than the normal cells, and were easily cleared. It is unlikely that the host defense functions would be as efficient for these filamentous cells in an abnormal physiological condition such as diabetes mellitus¹⁴⁻¹⁵). This may account for the poor therapeutic efficacy in diabetic mice. It has been reported that an important factor determining the efficacy of β -lactams is the total duration of the effective concentration¹⁰). Therefore, these antibiotics must be applied in large quantities and in multiple administrations. Moreover, regarding diabetes mellitus it is necessary to study chemotherapy in diabetic hosts. We are currently investigating these problems.

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