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Experimental Respiratory Tract Infection with *Klebsiella pneumoniae* DT-S in Mice: Chemotherapy with Kanamycin

TAKESHI NISHI AND KANJI TSUCHIYA*

Central Research Division, Takeda Chemical Industries, Ltd., Osaka, Japan

Such factors as suspending medium, operating pressure, exposure time, inoculum size, and strain, sex, age, and weight of the animals were examined for their effects on the development of respiratory tract infection with *Klebsiella pneumoniae* DT-S in mice. The suspending medium was one of the most important factors. Aerosol challenge with a 10^9 colony-forming units per ml resulted in deposition of 10^4 colony-forming units of the organisms in the lung. The numbers of organisms in the lung increased rapidly, and by 30 h, a well-developed pneumonia was apparent. All the mice died within 4 days after infection. The therapeutic effectiveness of single-dose kanamycin regimens decreased markedly with a delay in administration. The effectiveness of multi-dose kanamycin regimens was influenced by the frequency of dosage. Thus a 12-h dosage schedule was superior to a 24-h regimen. Administration of 20 mg of kanamycin per kg at 12-h intervals for 10 days, initiated 30 h after infection, provided a complete cure. The infecting organisms in the lung, trachea, and blood were eradicated by the kanamycin therapy, but those in the nasal cavity were difficult to eliminate.

Klebsiella pneumoniae has been recognized as one of the most important causative organisms of nosocomial infections, particularly in debilitated patients, and the pneumonia caused by this organism is often difficult to control (11, 19, 23, 24, 34). Mice challenged intraperitoneally are most commonly used to assess the activities of therapeutic agents against infection with this organism. To predict the clinical effectiveness of antimicrobial agents more adequately, however, it is desirable to test their activities against infections that more closely resemble the target disease (21). Although there have been numerous reports on the production and characteristics of experimental respiratory tract infections with *K. pneumoniae* (2, 7, 9, 13, 20, 22, 27-29, 31), only a few have been concerned with chemotherapeutic evaluations (2, 20, 28). Berendt et al. (2) recently reported a satisfactory murine model of pneumonia caused by this organism and the therapeutic effectiveness of kanamycin against the condition. In this model, however, a few untreated mice survived, and only the challenge organisms found in the lung and blood were followed. In the present report, several experimental conditions were examined to establish a uniform respiratory tract infection induced by *K. pneumoniae* in mice, and the effectiveness of kanamycin was studied in detail.

MATERIALS AND METHODS

Mice. The mice used in these studies included: 4- to 6-week-old males and 4-week-old females of the Slc:ICR strain (Shizuoka Agricultural Cooperative Associations for Laboratory Animals); 4-week-old females of the Slc:ddY strain (Shizuoka Agricultural Cooperative Associations for Laboratory Animals); 4-week-old males and females of the JCL:CF#1 strain (CLEA, Japan Inc.); 4-week-old males and females of the CF#1/H strain (Takeda Chemical Industries, Ltd.); and 4-week-old females of the CF#1/K strain (Takeda Chemical Industries, Ltd.). Four-week-old Slc:ICR male mice weighing 18 to 22 g were used primarily. Mice were caged in groups of 10 to 15 and given food and water ad libitum.

Organism. *K. pneumoniae* DT-S (biotype *edwardsii*, capsular type 1) was established from *K. pneumoniae* DT by the cloning method. After incubation at 37°C for 20 h on Trypticase soy agar (BBL Microbiology Systems), *K. pneumoniae* DT-S produces a characteristic glossy colony which trails strands of material. The colonies are circular and convex, have an average diameter of 0.5 mm, and are microscopically structureless. The biochemical reactions of *K. pneumoniae* DT-S are as follows: indole (−), methyl red (−), Voges-Proskauer (+), citrate (+), gelatinase (−), KCN (+), malonate (+), gas from glucose (−), gas from lactose (−), acid from dulcitol (−), and gluconate (+). The organism has been maintained by monthly transfer to Trypticase soy agar slant and stored at 4°C. In the experiments described here, a stock culture was first transferred onto a brain heart infusion agar slant (brain heart infusion [Difco], solidified by adding agar [Difco] to 1.5%), and incubated overnight at 37°C. Organisms from this culture were transferred to brain heart infusion broth, which was then incubated overnight at 37°C. The cells in this broth culture were sedimented by centrifuging at 12,000 × *g* for 20 min and were then suspended in phosphate-buffered saline,

pH 7.2 (PBS; $NaHPO_4$, 1.15 g; KH_2PO_4, 0.2 g; NaCl, 8.0 g; KCl, 0.2 g; distilled water, 1,000 ml). This suspension was again centrifuged, and the sedimented cells were suspended in appropriate amounts of PBS or brain heart infusion.

Respiratory tract infection procedure. The aerosol apparatus used for the infection is illustrated in Fig. 1. Up to 120 mice were placed in the exposure chamber (50 cm in diameter, 45 cm high) without restriction. The bacterial suspension, charged in a nebulizer (Vaponefrin Pocket Nebulizer; USV Pharmaceutical Co., Tuckahoe, N.Y.), was aerosolized with compressed air. This aerosol was drawn through the exposure chamber and then passed through five air-washing bottles connected to an aspirator. The first four air-washing bottles each contained 4 liters of 3% phenol solution, and the fifth contained 4 liters of water. The atmospheric pressure in the exposure chamber was maintained slightly lower (1.3 cm of water column) than the ambient atomospheric pressure. At the end of nebulization, fresh air was introduced into the exposure chamber. When a bacterial suspension of 10^9 colony-forming units (CFU) per ml was nebulized at an operating pressure of 1 kg/cm^2 for 40 min, the maximal numbers of organisms in the chamber air, about 500 CFU/ml, were reached 10 min after the start of nebulization, and this level was maintained. After the nebulizer was turned off, the number of bacteria in the chamber air declined rapidly, and after 30 min, no bacteria were detected. No bacteria were found in exhaust air throughout the aerosol trials. The bacterial content in the exposure chamber air or in the air that had passed through five air-washing bottles was determined by collecting multiple air samples at the rate of 15 ml per 10 s into syringes containing 5 ml of brain heart infusion broth. The syringes were then shaken violently 100 times. Colony counts were performed on the samples of brain heart infusion broth obtained in this manner.

Bacterial examination. Mice were killed with ether and bled from the axillary artery and vein. A 0.1-ml sample of this blood was inoculated onto a Trypticase soy agar plate. After the gross appearances of the viscera were noted, the cut surfaces of lung, liver, spleen, and kidneys were imprinted onto Trypticase soy agar plates, which were incubated overnight at 37°C. Quantitative assessments of bacterial populations were made, as follows. The lung and trachea were homogenized in 4 and 2 ml of distilled water, respectively, using a Teflon tissue homogenizer. The nasal cavity was washed with 2 ml of sterile distilled water, infused into the nasal passage from the choana and drained from the external nares. These homogenates and washings were serially diluted 10-fold with distilled water, and 0.1-ml volumes of the various dilutions were inoculated onto Trypticase soy agar plates and incubated at 37°C for 20 h. Colonies were counted and expressed as the log number of CFU per organ, or per milliliter of nasal washing or blood. When no bacteria were detected in undiluted specimens, a value of 0 was assigned to calculate the geometric mean titer.

Treatment. Kanamycin sulfate (Takeda Chemical Industries, Ltd., Osaka, Japan), dissolved in sterile 0.85% NaCl, was injected subcutaneously (0.2 ml per mouse) according to the schedules presented in Tables 5 and 6 and Figs. 3 to 5. The minimum inhibitory concentration of kanamycin against *K. pneumoniae* DT-S by the agar dilution method (33) is 1.56 μg/ml. The amount of kanamycin (milligrams per kilogram) required for 50% survival of the animals (50% survival dose) and that required for an eradication of challenge organisms from the lung of 50% of the animals tested (50% clearance dose), respectively, were calculated at the end of the observation period by the method of Reed and Muench (25) or by the probit method (18).

Histopathological examination. Specimens obtained from the animals were fixed in 10% neutral Formalin or Carnoy solution and embedded in paraffin. Sections were stained with hematoxylin and eosin, or by the periodic acid-Schiff technique, and examined under light microscopy.

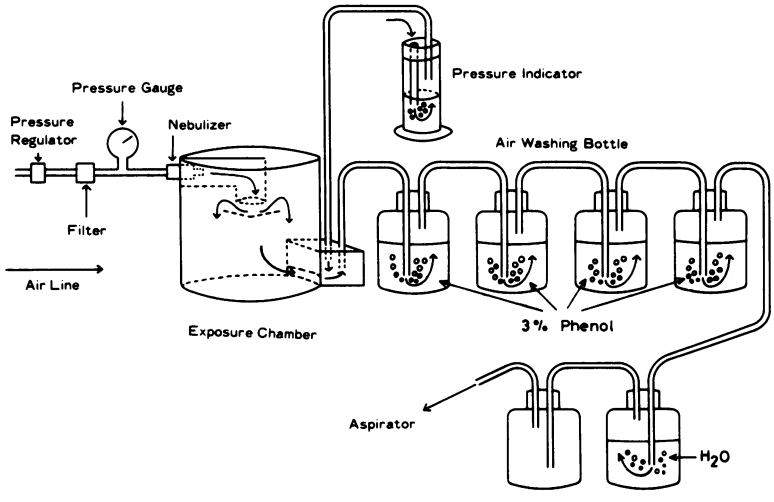

FIG. 1. *Aerosol infection apparatus.*

RESULTS

Factors affecting the course of infection. A bacterial suspension of 10^9 CFU/ml of PBS or brain heart infusion broth, charged in the nebulizer, was aerosolized at an operating pressure of 1 kg/cm^2 for 40 min (Table 1). Mice exposed to the PBS-based aerosol had over 10 times more bacteria in their lungs than those exposed to broth-based cloud. PBS was used as the suspending medium in the experiments described below. Differences in operating pressure over the range studied had no effect on the numbers of bacteria recovered from the lungs of the animals of each group. There was a constant rate of increase in the number of bacteria in the lungs during the exposure period of 40 min. A linear relation existed between the log number of bacteria in the nebulizer and that in the lungs of mice at the nebulizer concentration of 10^6 to 10^9 bacteria per ml. Exposure of mice to 10^9-CFU/ml generated aerosol resulted in a deposition of about 10^4 CFU per lung, and all the animals died within 4 days after exposure.

Marked mouse strain differences in susceptibility to the infection, as measured by mortality, were observed when 10^7 CFU of the organism per ml were nebulized (Table 2). Slc:ICR and CF#1/K mice showed high susceptibility to the infection, whereas JCL:CF#1 and CF#1/H were less susceptible. These differences largely disappeared with the 10^9-CFU/ml challenge. With a challenge dose of 10^7 CFU/ml there were sex differences in susceptibility in two of the three strains tested; in both strains (JCL:CF#1 and CF#1/H), males were more susceptible than females. No differences were observed in mortality over an age range of 3 to 6 weeks, although a trend toward an increase in survival time with increasing age was noted. With the 10^9-CFU/ml challenge, body weight over the range studied had no effect on either mortality or survival time.

Characteristics of the respiratory tract infection. Male Slc:ICR mice weighing 18 to 22 g were exposed for 40 min to aerosol generated from 10^9 CFU of *K. pneumoniae* DT-S per ml of PBS at an operating pressure of 1 kg/cm^2. The mice showed no signs of illness until 24 h after exposure. By 30 h the mice became weak, and thereafter they exhibited a steady downhill course; the death of the first animal occurred by 48 h, and most of the mice succumbed within 72 to 96 h. Although histological evidence of pneu-

TABLE 1. *Effects of several factors on respiratory tract infection with K. pneumoniae DT-S in mice*[a]

Factor	Suspension medium	Operating pressure (kg/cm^2)	Exposure time (min)	Nebulizer concn[b] (CFU/ml)	No. of bacteria in lung:[c] (log CFU in tissue; mean ± SD)		Mortality[d] (deaths/total)	Time to death[e] (days; mean ± SD)
Suspending medium	PBS	1.0	40	10^9	3.96 ± 0.21	(20)	80/80	3.1 ± 0.5
	BHI broth[f]	1.0	40	10^9	2.47 ± 0.38	(20)	80/80	3.7 ± 0.7
Operating pressure	PBS	0.6	40	10^9	3.89 ± 0.07	(10)	40/40	3.1 ± 0.5
	PBS	1.0	40	10^9	3.78 ± 0.11	(10)	40/40	3.2 ± 0.5
	PBS	1.4	40	10^9	3.83 ± 0.12	(10)	40/40	3.2 ± 0.5
Exposure time	PBS	1.0	5	10^9	2.67 ± 0.14	(10)	40/40	3.4 ± 0.9
	PBS	1.0	10	10^9	2.90 ± 0.25	(10)	40/40	3.1 ± 0.6
	PBS	1.0	20	10^9	3.26 ± 0.21	(10)	40/40	3.1 ± 0.5
	PBS	1.0	30	10^9	3.45 ± 0.26	(10)	40/40	3.0 ± 0.4
	PBS	1.0	40	10^9	3.64 ± 0.13	(10)	40/40	3.0 ± 0.4
Nebulizer concentration	PBS	1.0	40	0	0	(15)	0/60	
	PBS	1.0	40	10^5	0	(15)	1/60	12.0
	PBS	1.0	40	10^6	1.06 ± 0.34	(15)	15/60	4.8 ± 2.3
	PBS	1.0	40	10^7	2.05 ± 0.29	(15)	50/60	4.6 ± 1.2
	PBS	1.0	40	10^8	2.96 ± 0.22	(15)	60/60	3.9 ± 1.0
	PBS	1.0	40	10^9	4.05 ± 0.14	(15)	60/60	3.2 ± 0.5

[a] Four-week-old Slc:ICR male mice weighing 18 to 23 g were used. Bacterial suspension was nebulized at the indicated pressure for the period of time indicated.

[b] Bacterial suspension of 10^9 ($10^{8.89}$ to $10^{9.24}$) CFU/ml was used except in the nebulizer concentration study, in which serial 10-fold dilutions with PBS of the suspension ($10^{9.00}$ to $10^{9.20}$ CFU/ml) were used.

[c] Number of bacteria found in the lung of mice 15 min after completion of aerosol challenge. SD, Standard deviation. Parentheses indicate *n*.

[d] Experiments were terminated at 7 days after infection except in those in which the effect of nebulizer concentration was examined. In the latter, experiments were terminated at 13 days after infection.

[e] Time to death was calculated for fatal cases only.

[f] BHI, Brain heart infusion.

TABLE 2. *Effects of several mouse factors on respiratory tract infection with K. pneumoniae DT-S in mice*[a]

Nebulizer concn (CFU/ml)	Factor	Strain	Sex	Age (wks)	Wt (g)	Mortality[b] (deaths/total)	Time to death[c] (days; mean ± SD)
10^7	Strain	Slc:ICR	F	4	18–22	40/60	5.7 ± 2.7
		JCL:ICR	F	4	18–22	27/60	6.6 ± 2.5
		JCL:CF≠1	F	4	13–20	8/60	6.4 ± 2.9
		CF≠1/H	F	4	17–23	10/60	7.5 ± 3.0
		CF≠1/K	F	4	18–22	36/60	5.3 ± 2.7
		Slc:ddY	F	4	18–22	32/60	7.0 ± 3.1
10^7	Sex	Slc:ICR	M	4	18–22	48/60	4.7 ± 1.9
		Slc:ICR	F	4	18–22	43/60	5.5 ± 1.6
		JCL:CF≠1	M	4	13–18	32/60	5.4 ± 3.0
		JCL:CF≠1	F	4	13–18	13/60	5.6 ± 2.4
		CF≠1/H	M	4	17–23	34/60	6.6 ± 3.1
		CF≠1/H	F	4	17–23	16/60	6.6 ± 3.2
10^7	Age	Slc:ICR	M	3	10–14	45/60	4.4 ± 1.6
		Slc:ICR	M	4	19–21	43/60	5.0 ± 2.1
		Slc:ICR	M	5	25–28	41/60	6.0 ± 1.9
		Slc:ICR	M	6	28–32	43/60	6.3 ± 1.9
10^9	Strain	Slc:ICR	M	4	18–34	35/35	3.1 ± 0.5
		JCL:ICR	F	4	18–24	35/35	4.3 ± 0.9
		JCL:CF≠1	F	4	14–23	35/35	4.5 ± 1.9
		CF≠1/H	M	4	18–24	33/35	3.8 ± 1.4
		CF≠1/K	F	4	18–24	35/35	2.8 ± 0.5
		Slc:ddY	M	4	18–24	31/35	4.5 ± 2.9
10^9	Body wt	Slc:ICR	M	4	18–20	60/60	3.0 ± 0.4
		Slc:ICR	M	4	20–22	60/60	3.0 ± 0.4
		Slc:ICR	M	4	22–24	60/60	3.0 ± 0.4

[a] Bacterial suspension of 10^7 ($10^{6.90}$ to $10^{7.11}$) CFU/ml or 10^9 ($10^{8.89}$ to $10^{9.13}$) CFU/ml was nebulized at a pressure of 1 kg/cm^2 for 40 min.

[b] Mortality at 13 days after infection.

[c] Time to death was calculated for fatal cases only. SD, Standard deviation.

monia existed, no visible lesions were observed in the lung at 18 h after infection. A patchy distribution of reddish pinhead lesions, however, became visible in the lung by 24 h. Thereafter, the gross pulmonary lesions expanded progressively, and by 30 h, the lung had patchy consolidations and abscesses. The pleural cavities of mice examined 72 h after infection contained a viscous exudate. Representative microscopic changes of the lung at 30 and 72 h after infection are shown in Fig. 2.

Except for one animal, challenge organisms were found only in the lung during the first 24 h of infection but were distributed to the blood and other organs at later times (Table 3). The numbers of bacteria in the lung rapidly increased with time and reached about 10^5 times the initial level ($10^{8.6}$ to $10^{8.8}$ CFU per lung) at 36 to 40 h (Table 4). In the trachea, the bacterial count reached about 10^2 times the initial level ($10^{3.5}$ CFU per trachea) 18 h after exposure, and was maintained thereafter, at a level that was almost the same or slightly lower. Bacterial species other than *K. pneumoniae* also were frequently found in the trachea 18 to 48 h after aerosol challenge. The numbers of challenge organisms in the nasal washings remained unchanged throughout the observation period. In the blood, the organism first appeared at 24 h in one of five mice examined, and from 30 h onwards, all but one mouse were bacteremic.

Effect of kanamycin. (i) Single-dose therapy. The time effect of a single administration of kanamycin on the course of disease is summarized in Table 5. Although similar results were obtained with treatment at 3 or 6 h after infection, the activity of kanamycin decreased markedly with delay of administration. The 50% survival and 50% clearance doses (milligrams per kilogram) when treatment was delayed for 30 h were about 100 times as large as those for treatment delayed only 3 h. The incidence of grossly detectable pulmonary lesions roughly paralleled the occurrence of positive lung cultures. The numbers of bacteria in the respiratory organs and blood of mice after administration of a single dose of 1.25 or 5 mg of kanamycin per kg at 3 h after inoculation are presented in Fig. 3. Increases in numbers in lung, trachea, and blood were suppressed in proportion to the dose administered. Kanamycin at 5 mg/kg, which resulted in 90% survival at 6 days after infection,

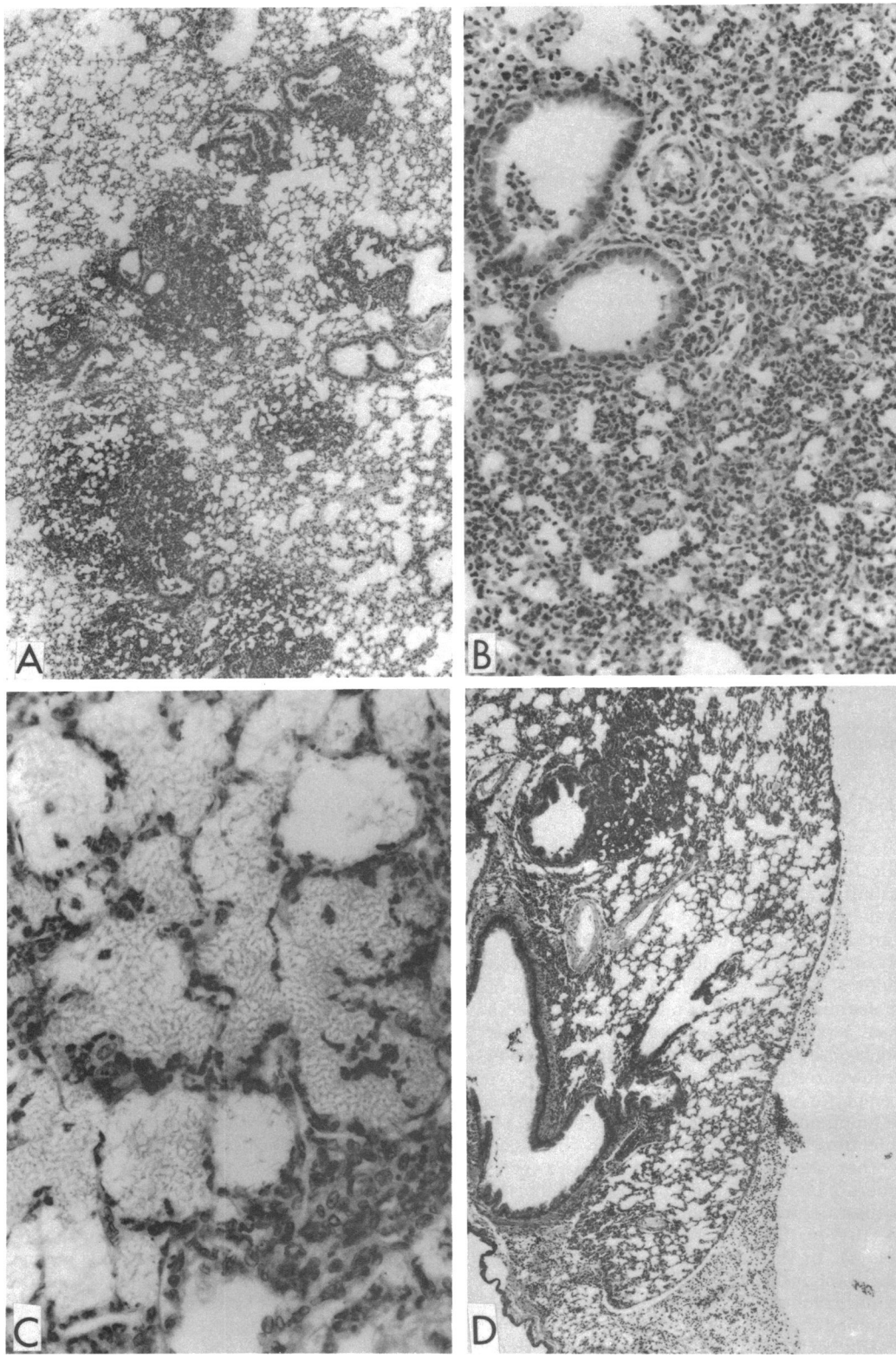

FIG. 2. *Histopathological changes in the lungs of mice infected with K. pneumoniae DT-S by aerosol method. (A) Bronchopneumonia at 30 h. Stained with hematoxylin and eosin. ×40. (B) Neutrophilic leukocyte infiltration in bronchiole and pulmonary alveoli at 30 h. Stained with hematoxylin and eosin. ×200. (C) Numerous bacilli in pulmonary alveoli at 30 h. Stained by periodic acid-Schiff technique. ×400. (D) Extension of polymorphonuclear leukocytes through pleural membrane at 72 h. Stained with hematoxylin and eosin. ×30.*

TABLE 3. *Distribution of K. pneumoniae DT-S to several organs of mice after aerosol infection*[a]

Time after infection (h)	Bacterial recovery from:[b]									
	Lung		Liver		Spleen		Kidney		Blood	
0	++	(10/10)	−	(0/10)	−	(0/10)	−	(0/10)	−	(0/10)
24	+++-++++	(10/10)	−	(0/10)	−-+	(1/10)	−	(0/10)	−-+	(1/10)
48	++++	(10/10)	−-+++	(8/10)	+-+++	(10/10)	+-+++	(10/10)	+-+++	(10/10)
72	++++	(10/10)	+++-++++	(10/10)	+++	(10/10)	++++	(10/10)	+++-++++	(10/10)

[a] Four-week-old Slc:ICR male mice weighing 18 to 22 g were used. Bacterial suspension ($10^{8.84}$ to $10^{9.06}$ CFU/ml) was nebulized at a pressure of 1 kg/cm^2 for 40 min.

[b] The cut surfaces of organs and 0.1-ml blood samples were imprinted and inoculated, respectively, onto Trypticase soy agar plates. After incubation at 37° C for 20 h, colonies were counted. The degree of bacterial recovery was expressed as follows: 0 CFU, −; 1 to 10 CFU, +; 11 to 100 CFU, ++; 101 to 1,000 CFU, +++; >1,000 CFU, ++++. Fractions in parentheses represent rates of positive bacterial recovery.

TABLE 4. *Bacterial populations in lung, trachea, nasal washings, and blood of mice infected with K. pneumoniae DT-S by aerosol method*[a] *(n = 5)*

Time after infection (h)	No. of bacteria (log CFU in tissue or per ml)			
	Lung	Trachea	Nasal washing	Blood
0	3.70 ± 0.12	1.87 ± 0.22	2.70 ± 0.22	0
6	4.20 ± 0.11	2.50 ± 0.18	2.49 ± 0.35	0
12	5.17 ± 0.31	2.80 ± 0.38	2.35 ± 0.54	0
18	6.15 ± 0.31	3.51 ± 0.23	2.41 ± 0.33	0
24	7.16 ± 0.35	3.15 ± 0.42	2.75 ± 0.32	1.17[b]
30	7.75 ± 0.23	2.66 ± 0.48	2.75 ± 0.27	1.51 ± 0.11[c]
36	8.60 ± 0.12	2.65 ± 0.70	3.03 ± 0.25	3.21 ± 0.70
42	8.78 ± 0.18	2.65 ± 0.41	3.02 ± 0.35	2.75 ± 0.67
48	8.83 ± 0.25	2.61 ± 0.44	2.89 ± 0.54	3.07 ± 0.55

[a] Four-week-old Slc:ICR male mice weighing 18 to 22 g were used. Bacterial suspension ($10^{8.97}$ to $10^{9.02}$ CFU/ml) was nebulized at a pressure of 1 kg/cm^2 for 40 min. Each value represents the mean ± standard deviation for positive cultures only. Unless otherwise mentioned, positive rate was 5/5.

[b] One positive culture.

[c] Four positive cultures.

cleared the challenge organisms from lung, trachea, and blood of mice within 48 h. Challenge organisms were still present in the nasal washings at this time.

(ii) Multiple-dose therapy. Two treatment schedules were followed. First, kanamycin was administered every 24 h for 3 to 10 days; second, it was administered every 12 h for 2 to 10 days. In both dosage schedules, the first dose of kanamycin was administered 30 h after infection. Measured by comparative 50% survival and 50% clearance doses, the 12-h regimen was about 1.5 times as effective as the 24-h schedule (Table 6). The data in Table 6 also show that the 12-h administration of 20 mg of kanamycin per kg ultimately sterilizes the lungs of all animals, whereas 24-h treatment at this level did not eradicate the challenge organism from the lung. To clarify the relationship between the therapeutic effectiveness of kanamycin and the dosage schedule, changes in bacterial numbers in the respiratory organs and blood of mice after a single administration of 20 mg of kanamycin per kg at 30 h after infection were followed over a 24-h period (Fig. 4). The bacterial numbers in the lung dropped to nearly one-tenth of the pretreatment level 6 h after dosing, were sustained at this level for 12 h, and thereafter gradually increased, almost reaching the pretreatment level 24 h after dosing. The numbers of bacteria in the trachea declined to one-hundredth of the pretreatment levels 6 h after dosing and increased thereafter. In contrast, bacterial numbers in nasal washings remained unchanged regardless of antibiotic administration. Challenge organisms disappeared rapidly from the blood 48 h after infection. Only one mouse was bacteremic. Subcutaneous administration of 20 mg of kanamycin per kg produces levels of 14.7 μg/g of lung at 15 min, 9.9 μg/g at 30 min, 3.2 μg/g at 1 h, and 0.6 μg/g at 2 h after dosing (16). These findings indicate that the numbers of bacteria in the lung are suppressed below the pretreatment level for 18 h, when levels of kanamycin in lung tissue are maintained above the minimum inhibitory concentration (1.56 μg/ml)

TABLE 5. *Effect of single-dose kanamycin therapy in mice infected with K. pneumoniae DT-S by aerosol method*[a]

Treatment[b]		Survival rate[c] (survival/total)	Time to death[d] (days; mean ± SD)	Rate of mice positive for:[e]		No. of bacteria in lung[f] (log CFU in tissue; mean ± SD)	50% effective dose (mg/kg) calculated by:[g]	
Time after infection (h)	Dose (mg/kg)			Gross lesion (positive/survival)	Bacterial recovery (positive/survival)		Survival rate (SD_{50})	Eradication rate (CD_{50})
3	1.25	0/20	4.6 ± 0.8					
	5	18/20	4.5 ± 0.7	0/18	4/18	1.71 ± 0.82	2.72	3.90
	20	20/20		0/20	3/20	2.13 ± 1.30		
6	1.25	1/20	4.2 ± 0.7	0/1	0/1			
	5	19/20	6.0	0/19	1/19	1.30	2.50	2.79
	20	20/20		0/20	2/20	2.16 ± 0.06		
18	5	6/20	5.2 ± 0.6	6/6	6/6	8.06 ± 1.26		
	20	9/20	5.7 ± 0.5	7/9	7/9	7.11 ± 0.89		
	80	16/20	5.5 ± 0.6	7/16	6/16	7.26 ± 0.35	21.1	74.6
	320	19/20	6.0	0/19	0/19			
24	5	1/20	4.5 ± 0.5	1/1	1/1	8.17		
	20	9/20	5.5 ± 0.5	9/9	9/9	8.23 ± 1.14		
	80	15/20	5.6 ± 0.9	13/15	13/15	7.07 ± 1.36	33.9	>320
	320	17/20	5.3 ± 0.6	10/17	10/17	7.24 ± 0.73		
30	20	0/20	4.8 ± 0.5					
	80	0/20	5.3 ± 0.7				>320	>320
	320	5/20	5.3 ± 0.9	5/5	5/5	7.57 ± 0.89		
Infected control		0/20	3.2 ± 0.5					

[a] Four-week-old Slc:ICR male mice weighing 18 to 22 g were used. Bacterial suspension ($10^{8.89}$ to $10^{9.05}$ CFU/ml) was nebulized at a pressure of 1 kg/cm^2 for 40 min.

[b] Kanamycin was administered subcutaneously in a single dose at the indicated time after infection.

[c] Survival rate at 6 days after infection.

[d] Time to death was calculated for fetal cases only. SD, Standard deviation.

[e] Animals that survived were killed 6 days after infection and examined for gross pulmonary lesions and bacterial recovery from the lung.

[f] Number of bacteria in lung was calculated for positive lungs only.

[g] 50% effective dose was calculated by the method of Reed and Muench. SD_{50}, 50% survival dose; CD_{50}, 50% clearance dose.

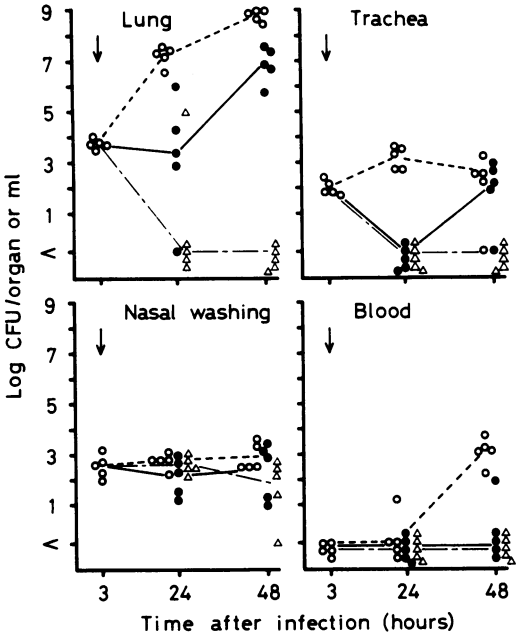

FIG. 3. *Bacterial numbers in the respiratory organs and blood of mice after a single subcutaneous administration of kanamycin at 3 h after infection. Four-week-old Slc:ICR male mice weighing 18 to 24 g were infected by the aerosol method, in which bacterial suspension ($10^{8.96}$ to $10^{8.987}$ CFU/ml) was nebulized at a pressure of 1 kg/cm^2 for 40 min. Each point (○, untreated; ●, 1.25 mg/kg; △, 5 mg/kg) represents the value for a mouse. Arrow indicates time of medication, and < indicates below the level of detectability (40 CFU per lung; 20 CFU per trachea; 10 CFU/ml of nasal washing or blood).*

for about 2 h. The bacterial numbers in the respiratory organs and blood of mice treated with 20 mg of kanamycin per kg at 12-h intervals were further examined daily (Fig. 5). The challenge organism first disappeared from the blood, then from trachea and lung; eradication from the nasal cavity was difficult.

DISCUSSION

The suspending medium was by far the most important factor affecting the course of infection in this study. Laurenzi et al. (17) reported that a suspension of staphylococci in buffer was superior to suspensions in broth for aerosol infection. Similar results were obtained in the present study using *K. pneumoniae* DT-S as a challenge organism. It is speculated that a greater number of aerosol particles with diameters of about 1 μm, shown to be deposited in large part in the depth of the lower respiratory tracts of animals (12, 35), were generated from PBS suspension than from broth suspension. Berendt (1) reported that *K. pneumoniae* was more virulent when given by intranasal instillation than when given by small-particle aerosols. However, equivalent doses of *K. pneumoniae* DT-S given to mice by either route produced similar responses (unpublished data). The 50% lethal doses of *K. pneumoniae* DT-S for Slc:ICR mice is less than 10^2 CFU per lung by aerosol infection and 10^4 CFU per mouse by intraperitoneal infection (unpublished data), showing that *K. pneumoniae* DT-S is more virulent by respiratory tract infection. It is well known that various animal variables such as species, strain, sex, age, body weight, and breeding conditions should be considered in establishing experimental infections (3, 10, 32). In aerosol infection with *K. pneumoniae* in mice, Ehrlich (7) reported that there were strain differences in susceptibility to infection, though no differences were observed in mortality among animals ranging in age from 6 weeks to 9 months. In the present study, a marked strain difference in susceptibility to the aerosol infection with *K. pneumoniae* DT-S was observed; male mice were more susceptible than females in two of three strains tested.

Branch and Stillman (4) reported that pulmonary lesions produced in mice after inhalation of Friedländer's baccilus resembled pneumonia caused by this organism in humans. In the present study, characteristic features of *K. pneumoniae* pneumonia in humans, such as abscess formation in the lung and rapid progress of the disease with high mortality (26), were observed in mice infected with *K. pneumoniae* DT-S by the aerosol method. Furthermore, histologically, the pneumonic lesions of mice had several features in common with pneumonias occurring in humans. Besides a marked growth of *K. pneumoniae* DT-S in the lung, an obvious increase in the number of challenge organisms was observed in the trachea. Whether this increase was due to bacterial multiplication in situ or to transportation of the organisms from the lung via mucociliary movement (5) is not clear. *Staphylococcus aureus* colonized in the human upper respiratory tract is reportedly difficult to eliminate by oral antibiotics (36). Gram-negative bacteria, including *K. pneumoniae*, colonized in the upper respiratory tract of debilitated patients, especially those undergoing intensive antibacterial or anticancer chemotherapy, frequently produce a fatal pneumonia (14). Hamsters reportedly develop a high incidence of *Klebsiella* pneumonia when the pharyngeal bacterial flora is modified by an antibiotic (6). On the other hand, the etiological significance of large numbers of *K. pneumoniae* isolated from human respiratory

TABLE 6. *Effect of multiple-dose kanamycin therapy in mice infected with K. pneumoniae DT-S by aerosol method*[a]

Treatment[b]			Survival rate[c] (survivals /total)	Time to death[d] (days; mean ± SD)	Rate of mice positive for:[e]		No. of bacteria in lung[f] (log CFU in tissue; mean ± SD)	50% effective dose (mg/kg) calculated by:[g]	
Interval	Dose (mg/kg)	No. of treatments			Gross lesion (positive /survival)	Bacterial recovery (positive /survival)		Survival rate (SD_{50})	Eradication rate (CD_{50})
24 h	20	3	2/20	7.6 ± 2.1	2/2	2/2	5.57 ± 1.12	111 (93.3–140)	>200 (190–>200)
		5	8/20	9.3 ± 2.0	5/8	6/8	7.61 ± 1.35		
		7	14/20	8.5 ± 2.7	12/14	7/14	5.86 ± 1.62		
		10	14/20	6.8 ± 2.3	11/14	9/14	5.80 ± 1.10		
24 h	40	3	8/20	8.3 ± 1.4	7/8	7/8	6.15 ± 1.40	133 (103–154)	189 (165–214)
		5	17/20	10.7 ± 2.1	5/17	5/17	6.05 ± 1.12		
		7	20/20		2/20	2/20	3.27 ± 2.78		
		10	20/20		5/20	2/20	2.89 ± 2.12		
12 h	10	3	1/20	7.5 ± 1.5	1/1	1/1	3.60	82.2 (69.2–97.7)	131 (115–154)
		5	5/20	8.0 ± 2.4	5/5	5/5	5.02 ± 0.80		
		7	7/20	10.2 ± 1.7	5/7	5/7	6.34 ± 1.13		
		9	11/20	9.9 ± 2.0	9/11	10/11	5.65 ± 1.41		
		13	16/20	11.3 ± 1.3	4/16	4/16	6.15 ± 1.35		
		19	18/20	8.5 ± 0.7	1/18	2/18	5.75 ± 2.88		
12 h	20	3	7/20	6.6 ± 1.7	4/7	4/7	5.54 ± 1.59	83.7 (62.0–100)	116 (96.6–135)
		5	10/20	9.2 ± 1.5	4/10	4/10	6.66 ± 1.82		
		7	16/20	11.0 ± 2.0	1/16	2/16	4.38 ± 3.30		
		9	19/20	11.0	0/19	4/19	2.27 ± 1.29		
		13	20/20		1/20	1/20	4.82		
		19	20/20		0/20	0/20			
Infected control			0/40	3.1 ± 0.6					

[a] Four-week-old Slc:ICR male mice weighing 18 to 23 g were used. Bacterial suspension ($10^{8.95}$ to $10^{9.11}$ CFU/ml) was nebulized at a pressure of 1 kg/cm^2 for 40 min.

[b] Subcutaneous administration of kanamycin was started at 30 h after infection, followed by doses at 24-h or 12-h intervals thereafter.

[c] Survival rate at 13 days after infection.

[d] Time to death was calculated for fatal cases only. SD, Standard deviation.

[e] Animals that survived were killed 13 days after infection and examined for gross pulmonary lesions and bacterial recovery from the lung.

[f] Number of bacteria in lung was calculated for positive lungs only.

[g] 50% effective dose (expressed as total dose) was calculated by the probit method. Numbers in parentheses indicate 95% confidence limits. SD_{50}, 50% survival dose; CD_{50}, 50% clearance dose.

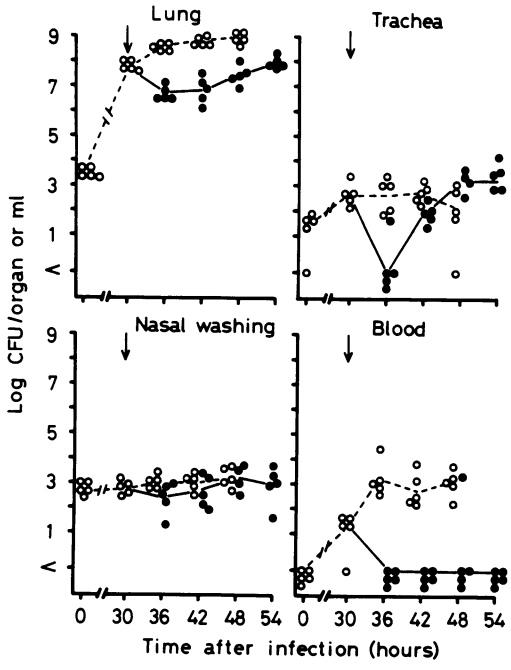

FIG. 4. *Bacterial numbers in respiratory organs and blood of mice after a single subcutaneous administration of kanamycin at 30 h after infection. Four-week-old Slc:ICR male mice weighing 18 to 24 g were infected by the aerosol method, in which bacterial suspension ($10^{8.97}$ to $10^{9.07}$ CFU/ml) was nebulized at a pressure of 1 kg/cm² for 40 min. Each point (○, untreated; ●, 20 mg/kg) represents the value for a mouse. Arrow indicates the times of medication, and < indicates below the level of detectability (40 CFU per lung; 20 CFU per trachea; 10 CFU/ml of nasal washing or blood).*

tract specimens has not been established (8). Considering these observations, the persistence of *K. pneumoniae* DT-S in the nasal cavity of mice after kanamycin therapy deserves further study.

The therapeutic effectiveness of single-dose kanamycin regimens was conditioned by the location of the infectious process at the time of therapy. If treatment was delayed until 30 h, a dose as large as 320 mg/kg produced only a marginal effect. Thirty hours after infection was selected as the time of initiation of multiple-dose therapy, based on the above observation as well

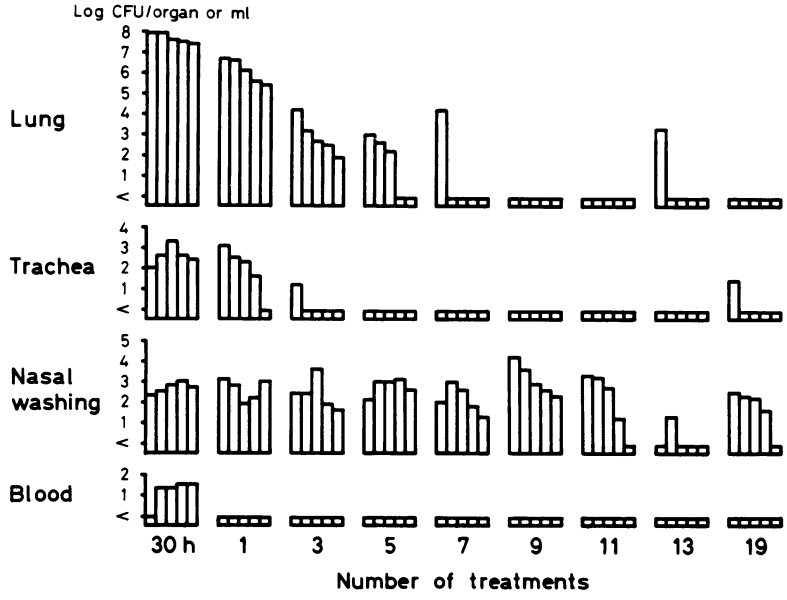

FIG. 5. *Bacterial numbers in the respiratory organs and blood of mice after multiple-dose therapy with 20 mg of kanamycin per kg. Four-week-old Slc:ICR male mice weighing 18 to 22 g were infected by the aerosol method, in which bacterial suspension ($10^{8.97}$ to $10^{8.99}$ CFU/ml) was nebulized at a pressure of 1 kg/cm² for 40 min. Subcutaneous administration of kanamycin was started at 30 h after infection, followed by doses at 12-h intervals. Bacterial examination was carried out 12 h after administration. Each bar represents the number of bacteria for a mouse, and < indicates below the level of detectability (40 CFU per lung; 20 CFU per trachea; 10 CFU/ml of nasal washing or blood).*

as for the following reasons: first, at 30 h, all animals had well-developed pneumonias, and second, the primary sites of infection at this time were the respiratory organs, although low levels of bacteremias were observed. The 12-hourly administration of kanamycin was superior to the 24-hourly or single-dose treatment. Since only three dosage regimens were tested, the most effective schedule for delivery of kanamycin requires further investigation. In the treatment of experimental pneumococcal pneumonia in rats, sodium penicillin is considerably more effective when administered at 8-h intervals than when given in either more frequent or more widely spaced doses (30). *Klebsiella* pneumonias in humans are often difficult to treat, and therapy is required for a minimum of 10 to 14 days (34). The murine pneumonia caused by *K. pneumoniae* DT-S also proved difficult to treat. It is important that a dosage regimen comparable to that employed clinically (15, 34) was therapeutically effective. These results suggest that the model infection used may be useful in assessing the effectiveness of antimicrobial agents in clinical use.

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