INFECTION AND IMMUNITY, May 1978, p. 581-583
0019-9567/78/0020-0581$02.00/0

Vol. 20, No. 2
Printed in U.S.A.

Relationship of Method of Administration to Respiratory Virulence of *Klebsiella pneumoniae* for Mice and Squirrel Monkeys

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Received for publication 21 December 1977

Klebsiella pneumoniae given by aerosol was significantly less virulent in mice and monkeys than when given by intranasal (mice) or intratracheal (monkeys) instillation.

Recently, we have published the details of two models for the study of respiratory *Klebsiella pneumoniae* infection: infection of mice after inhalation of small aerosol particles (3) and response of rats to intranasal (i.n.) instillation of the test organism (1). Recently, it became apparent that mice challenged by i.n. instillation died in greater numbers and earlier than did those given the same challenge dose by aerosol.

Because this observation raised the question of how well our models simulated the means by which the disease was acquired, we investigated the effect of the two routes in greater detail. We extended the investigation of *Klebsiella* infection to include infection of the squirrel monkey and compared aerosol with intratracheal instillation.

The methods of culture preparation, small-particle aerosol dissemination (median diameter, 2.2 μm), particle sizing, sampling, assay, and dose estimation have been described (3). The technique for i.n. inoculation of mice was the same as that described for rats (1), except that the inoculum volume was reduced to 0.05 ml. The method for dissemination of large aerosol particles has been described by Young et al. (9). The intratracheal instillation procedure for squirrel monkeys has also been described (2). Median lethal doses were calculated by the method of Litchfield and Wilcoxon (8).

The responses of mice to selected doses of *Klebsiella* given i.n. or by small- or large-particle aerosols are given in Table 1. Twenty-five mice were challenged at each of five dose levels. *K. pneumoniae* was approximately 75 times more virulent when it was given i.n. than when it was given by small-particle aerosols. No deaths were seen in mice given 2,000 organisms by large-particle aerosol, indicating that the organism was even less virulent by this method of challenge.

Failure of the aerosol particles to penetrate to airways in the lungs seemed a reasonable explanation for the lack of response in mice exposed to 7.0-μm aerosols, since it is known that 7.0-μm particles do not penetrate small airways (5). To determine whether lung penetration was a necessary prerequisite for infection, an experiment was performed in which 5×10^6 organisms were administered i.n. in 0.001 ml. Studies by Larson have indicated that influenza virus given to mice in this volume is not deposited in the lung but remains in the upper respiratory tract (E. W. Larson, personal communication). No mortality was observed in this experiment (Table 1), suggesting that deposition of *K. pneumoniae* in the upper respiratory tract was insufficient to establish infection.

An experiment was designed to determine whether the high median lethal dose of small-particle aerosols was due to loss of virulence caused by aerosolization. Groups of 25 mice were exposed to graded doses of *K. pneumoniae* in aerosols, or to i.n. instillation, and, in addition, were instilled i.n. with organisms collected from aerosols with impingers. The results indicated that aerosolization did not cause loss of virulence (Table 2).

Aerosol exposure of squirrel monkeys to doses as high as 10^7 cells caused no discernible response (Table 3). When intratracheal doses were administered in 0.5-ml volumes, all doses of 3×10^4 or greater were fatal; 3×10^2 organisms caused very mild illness (lethargy, anorexia) of no more than 3 days in duration. Increasing the volume of challenge from 0.5 to 1.0 ml and then to 1.5 ml increased the severity of illness as well as the percent mortality (Table 3). Finally, a dose-response experiment using 1.5 ml as a challenge volume was performed, and 50% of the monkeys died at a dose of 700 organisms. Illness lasting 5 to 7 days was characterized by fever, anorexia, dyspnea, and weight loss, and increased respiratory rate was observed in survi-

TABLE 1. *Virulence of K. pneumoniae for mice after i.n. or aerosol challenge*

Parameter	Vol (ml)	Median particle diam (μm)	LD_{50} (organisms)[a]	P[b]
Route of inoculation				
i.n.	0.05		17.9[c] ± 6.2	<0.025
SPA[d]		2.2	1,470 ± 506	
LPA[e]		7.0	No response at 2×10^3 cells	
Effect of inoculum volume (i.n.)				
i.n.	0.05		14.0	
i.n., small volume	0.001		No response at 5×10^6 cells	

[a] Mean ± standard error of five replicate determinations. LD_{50}, Median lethal dose.
[b] Probability was calculated by Student's t test against mice given small-particle aerosol.
[c] Calculated mean times to death of mice at a median lethal dose were 5.2 and 6.2 days for aerosol- and i.n. challenged mice, respectively.
[d] SPA, Small-particle aerosols.
[e] LPA, Large-particle aerosols.

TABLE 2. *Effect of aerosolization on the i.n. virulence of K. pneumoniae for mice*

Route of inoculation	Vol (ml)	Median particle diam (μm)	LD_{50} (organisms)[a]	P[b]
Aerosol		2.2	2,020 ± 689.0	
i.n., control	0.05		18.0 ± 8.3	<0.025
Aerosolized cells collected and given i.n.	0.05		19.2 ± 11.1	<0.025

[a] Mean ± standard error of five replicate determinations. LD_{50}, Median lethal dose.
[b] Probability was calculated by Student's t test against mice given small-particle aerosol.

vors (Table 3). Bacteria were isolated from the nasopharynx for 5 days. Persistent bacteremia (>2 days) was usually followed by death.

These observations must be considered by anyone who wishes to establish respiratory infection in experimental animals. The difference in response to aerosol and i.n. instillation does not occur with all microorganisms. For example, Larson et al. have shown that equivalent doses of influenza virus given to mice by either route produce similar responses (7). Also important is the question of whether the diseases established by different methods of administration differ significantly in pathogenesis. This subject is cur-

TABLE 3. *Effect of dose and inoculum volume on reaction of squirrel monkeys to intratracheal or aerosol challenge*

Determination and route of challenge	Dose (cells)	Inoculum vol (ml)	No. of monkeys	Response
Dose response				
Aerosol	2×10^2		2	None detectable
	2×10^3		2	
	3×10^4		2	
	2×10^5		2	
	3×10^6		4	
	1×10^7		6	
Intratracheal	3×10^2	0.5	2	Mild illness, 2- to 3-day duration
	3×10^4		2	Two dead, 30–48 h
	3×10^6		2	Two dead, 30–48 h
	3×10^8		2	Two dead, 30–48 h
Effect of volume of inoculum				
Intratracheal	3×10^3	0.5	4	Mild illness, no deaths
	3×10^3	1.0	4	All dead, MTD[a] = 40 h
	3×10^3	1.5	4	All dead, MTD = 40 h
Dose response (1.5-ml volume)				
Intratracheal	7×10^1	1.5	4	Transient, mild illness
	7×10^2	1.5	4	Two dead, two ill and recovered
	7×10^3	1.5	4	Two dead, MTD = 40 h

[a] MTD, Mean time to death.

rently under study.

It is tempting to speculate on a possible relationship of *K. pneumoniae* infection in mice and monkeys to human disease. If humans respond to *K. pneumoniae* as do monkeys, then one can say that infection with this organism does not occur as a result of inhalation of aerosols. This observation is consistent with the statement of Johanson et al. that gram-negative pneumonia occurs when hospital patients aspirate material from the pharynx (6).

It is difficult to explain the mechanism of our results. The effect of increasing volumes of instilled material on monkeys is probably due to increased resistance to lung clearance mechanisms or wider dispersion of organisms within the lungs. The number of bacteria found in the lungs of mice after i.n. or aerosol exposure was approximately equal (unpublished observation). However, organisms contained in small-aerosol particles of the size range we used reach the lower airways in the lungs (4, 5, 7), and it is possible that i.n. or intratracheally instilled organisms do not penetrate as deeply. If this is the case, then the aerosol-administered organisms may be more susceptible to host defenses such as phagocytosis than are bacteria deposited primarily in the larger airways in the lungs. This would be especially important during the early stages of infection. It is hoped that investigations in progress will resolve the question of mechanisms.

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