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RELATIONSHIP OF METHOD OF ADMINISTRATION TO RESPIRATORY VIRULEN--ETC(U)
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Running head: RESPIRATORY KLEBSIELLA INFECTION

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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

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ABSTRACT

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 following inhalation of small-aerosol particles (1) and response of rats to intranasal instillation (i.n.) of the test organism (3). 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 (1). The technique for i.n. inoculation of mice was the same as that described for rats (3) 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 (LD_{50}) were calculated by the method of Litchfield and Wilcoxon (8).

The responses of mice to selected doses of Klebsiella given i.n., by small-particle 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 in small-particle aerosols. No deaths were seen in mice given 2000 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 LD_{50} 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 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 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, weight loss, and increased respiratory rate was observed in survivors (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 has 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 currently 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 that we employed 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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TABLE 1. Virulence of K. pneumoniae for mice after i.n. or aerosol challenge

Route of inoculation	Volume (ml)	Median particle diameter (μm)	LD_{50} (organisms)[a] Mean ± SEM	P[b]
Intranasal instillation vs small-particle (SPA) and large-particle aerosols (LPA):				
i.n.	0.05		17.9[c] ± 6.2	<.025
SPA	-	2.2	1470 ± 506	-
LPA	-	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 ± SEM of five replicate determinations.

[b]Probability calculated by Student's t test against mice given small-particle aerosol.

[c]Calculated mean times to death of mice at an LD_{50} dose were 5.2 and 6.2 days for aerosol and i.n. challenged mice, respectively.

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

Route of inoculation	Volume (ml)	Median particle diameter (μm)	LD_{50} (organisms)[a] Mean ± SEM	P[b]
Aerosol	-	2.2	2020 ± 689.0	-
i.n.-control	0.05	-	18.0 ± 8.3	<.025
Aerosolized cells collected and given i.n.	0.05	-	19.2 ± 11.1	<.025

[a]Mean ± SEM of five replicate determinations.

[b]Probability calculated by Student's t test against mice given small-particle aerosol.

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

Route of challenge	Dose (cells)	Inoculum volume (ml)	No. of monkeys	Response
Dose Response:				
Aerosol	2×10^2	-	2	None detectable
	2×10^3	-	2	
	3×10^4	-	2	
	3×10^5	-	2	
	3×10^6	-	4	
	1×10^7	-	6	
Intra-tracheal	3×10^2	0.5	2	Mild illness, 2 to 3 day duration
	3×10^4		2	2 dead, 30-48 h
	3×10^6		2	" " "
	3×10^8		2	" " "
Effect of volume of inoculum:				
Intra-tracheal	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):				
Intra-tracheal	7×10^1	1.5	4	Transient, mild illness
	7×10^2	1.5	4	2 dead, 2 ill and recovered
	7×10^3	1.5	4	4 dead, MTD = 40 h

[a]MTD = mean time to death.

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