Pulmonary Absorption and Retention of Kanamycin
After Repeated Inhalation Administration
of Kanamycin Aerosol

RICHARD H. TESKE AND STEPHEN B. MILLER
Hill Top Research, Inc., Miamiville, Ohio

Received for publication 1 December 1969

The absorption and retention of kanamycin by the pulmonary system was evaluated after aerosol administration to rats. Tissue level concentrations of the drug were assayed by the agar-plate bioassay method. Mean concentrations of 6.58 µg/g and 3.52 µg/g were found in the lung and kidney homogenates, respectively, 26 hr after the final exposure period. No detectable level was found in the plasma.

There is considerable interest in aerosol therapy as a method of administration for a wide variety of therapeutic agents. Of special interest is the aerosol administration of antibiotics in broncho-pulmonary diseases.

Bilodeau et al. reported on kanamycin, an antibiotic active against staphylococci and many gram-negative organisms, in the treatment of suppurative bronchopulmonary diseases in humans, and commented briefly on the absorption and retention of kanamycin in the pulmonary system. Prokhorova reported the results of an investigation on the absorption of kanamycin after aerosol administration to rats (4). However, this information is published in Russian and thus is not readily accessible.

This study was undertaken to evaluate the absorption and retention of kanamycin by the pulmonary system after administration in aerosol form.

MATERIALS AND METHODS

The kanamycin used in this study was prepared as a solution of kanamycin sulfate injection (Kantrex Injection, Bristol Laboratories) in physiological saline (Sodium Chloride Injection, USP, Abbott Laboratories).

Method of administration. Thirty male Sprague-Dawley-derived rats (Laboratory Supply Co.) were exposed to an aerosolized kanamycin solution in an inhalation chamber once daily for 30 consecutive days. The dose of kanamycin each animal received can be determined by the formula of Prokhorova (4) as follows: \( X_p = \frac{0.5 \times C \times T}{(V \times t)} \), where \( X_p \) = dose of kanamycin in milligrams per kilogram of body weight, \( 0.5 \) = a constant coefficient, \( C = \) concentration of kanamycin in milligrams per milliliter of solution, \( T = \) duration of exposure in minutes, \( V = \) air flow in liters per minute, and \( t = \) time required to aerosolize 1 ml of solution in minutes.

The concentration of kanamycin, duration of exposure, and air flow were standardized at 30 mg/ml, 60 min, and 20 liters/min, respectively. The average dose of kanamycin per exposure was 15.7 mg/kg of body weight with 90% of the exposure resulting in dosage levels between 15.0 and 18.0 mg/kg of body weight.

The exposure chamber consisted of a stainless-steel cylinder with dimensions of 29.5 by 17.5 cm. One end of the chamber was made of plexiglass to permit observation of the animals, which were contained in expanded metal cages during exposure. The air input tube was positioned at the top of the chamber with the air exit tube at the bottom.

The aerosol of kanamycin solution was produced by an atomizer-reservoir assembly placed above the animals in the chamber.

At the conclusion of each daily exposure period, the animals were removed from the chamber and housed in groups in wire-mesh cages suspended above the droppings. Food, consisting of commercial pellets, and water were available to the animals ad lib.

The rats were sacrificed approximately 26 hr after the last exposure; blood samples, lungs, and kidneys were removed for analysis.

Preparation of samples. The blood samples were added to heparinized tubes and plasma obtained by centrifugation. Plasma was diluted 4X in sterile phosphate buffer (pH 7.9 ± 0.01) and stored at -40 C until assay.

Kidneys and lungs were weighed and then homogenized in a glass tissue grinder with a Teflon pestle. Phosphate buffer (pH 7.9) had been added to give a 4X dilution of tissue weight to volume. These homogenates were frozen at -40 C until asssay.
Agar plate bioassay method. The kanamycin level was assayed by the agar plate diffusion method with the use of Bacillus subtilis by a technique adapted from Grove and Randall (3).

Medium, diluents, and inoculum, standard preparation. The medium used in both base and seed layers in this procedure was Penassay Base Medium (Difco). The pH of the medium was adjusted to 7.9 ± 0.01 after sterilization: 15 ml of base layer, 6 ml of seed layer. The only diluent used was sterile phosphate buffer, pH 7.9 ± 0.01. The inoculum was prepared (Difco) spore suspension of B. subtilis ATCC 6633 and was added to the seed layer at 1.0 ml of suspension to 100 ml of agar. A 1,000 μg/ml solution of kanamycin in phosphate buffer was made from bulk powder supplied by Bristol Laboratories (lot no. B7484). From this solution, standards of 0.6, 0.8, 1.0, 1.2, 1.5, and 2.0 μg/ml were prepared.

Standard curve. Two plates were used for each concentration on the standard curve. Three Difco sterile paper concentration discs [0.25 inch (0.64 cm)] were wetted in the reference-point concentration (1.0 μg/ml) and three were wetted with the concentrations prepared from samples. The discs were then placed on the seeded plates so that each plate had six reference discs placed in alternating positions with six discs with unknown potency. The plates were incubated for 16 to 18 hr at 37 C. The diameters of the zones of inhibition were then measured to the nearest 0.2 mm by using a Vernier caliper.

The average zone diameters of all of the reference-point concentrations were averaged to give a standard zone diameter (SZ). The reference point concentrations on the individual sets of plates were averaged and compared to the SZ to provide a plus or minus correction factor with which to adjust the computed average of the zone diameters being determined. These adjusted values were then used to construct a standard curve by plotting kanamycin concentration (micrograms per milliliter) versus zone diameter (millimeters) on 2-cycle semi-logarithmic graph paper.

Tissue level determinations. The procedure used was the same as outlined above, except that appropriate samples of plasma, lung, or tissue homogenate were used instead of known concentrations. The average zone of inhibition of each sample of plasma, lung, or kidney was computed and adjusted to the SZ.

Computation of tissue level concentration. The level of kanamycin represented by the individual zone diameters was determined from the standard curve. This figure was then multiplied by the dilution factor to determine tissue level concentration. (Tissue level concentrations in homogenates were in terms of micrograms per gram of tissue.)

RESULTS

Table 1 is a tabulation of the findings obtained in this study. Tissue level concentrations of kanamycin were calculated by multiplying the adjusted zone diameter-standard curve reading by the dilution factor (4X).

| Animal no. | Kidney | Lung |
|------------|--------|------|
|            | Adjusted avg zone diameter | Standard curve reading | Tissue level concn | Adjusted avg zone diameter | Standard curve reading | Tissue level concn |
| mm | μg/s | mm | μg/s |
| mm | μg/s | mm |
| 1 | 0.85 | 0.58 | 2.32 | 1.19 | 1.37 | 5.48 |
| 2 | 0.98 | 0.81 | 3.24 | 1.09 | 1.07 | 4.28 |
| 3 | 0.99 | 0.83 | 3.32 | 1.29 | 1.77 | 7.08 |
| 4 | 1.00 | 0.85 | 3.40 | 1.09 | 1.07 | 4.28 |
| 5 | 1.15 | 1.25 | 5.00 | 1.41 | 2.40 | 9.60 |
| 6 | 1.19 | 1.37 | 5.48 | 1.59 | 3.75 | 15.00 |
| 7 | 1.00 | 0.85 | 3.40 | 1.12 | 1.15 | 4.60 |
| 8 | 0.88 | 0.63 | 2.52 | 1.33 | 1.95 | 7.80 |
| 9 | 0.94 | 0.94 | 3.76 | 1.28 | 1.75 | 7.00 |
| 10 | 1.18 | 1.35 | 5.60 | 0.91 | 0.67 | 2.68 |
| 11 | 0.91 | 0.67 | 2.68 | 1.19 | 1.36 | 5.44 |
| 12 | 0.98 | 0.81 | 3.24 | 1.30 | 1.85 | 7.40 |
| 13 | 0.99 | 0.83 | 3.32 | 0.93 | 0.71 | 2.84 |
| 14 | 0.91 | 0.67 | 2.68 | 0.91 | 0.67 | 2.68 |
| 15 | 1.00 | 0.85 | 3.40 | 0.93 | 0.71 | 2.84 |
| 16 | 1.16 | 1.27 | 5.08 | 1.36 | 2.10 | 8.40 |
| 17 | 1.06 | 1.00 | 4.00 | 1.20 | 1.45 | 5.80 |
| 19 | 0.99 | 0.83 | 3.32 | 1.16 | 1.27 | 5.08 |
| 20 | 0.88 | 0.63 | 2.52 | 1.29 | 1.77 | 7.08 |
| 21 | 1.06 | 1.00 | 4.00 | 1.23 | 1.52 | 6.08 |
| 22 | 0.94 | 0.73 | 2.92 | 1.36 | 2.10 | 8.40 |
| 23 | 0.71 | 0.41 | 1.64 | 1.26 | 1.70 | 6.80 |
| 24 | 1.14 | 1.20 | 4.80 | 1.36 | 2.10 | 8.40 |
| 25 | 1.01 | 0.87 | 3.48 | 1.26 | 1.70 | 6.80 |
| 26 | 0.91 | 0.67 | 2.68 | 1.13 | 1.20 | 4.80 |
| 27 | 1.06 | 1.00 | 4.00 | 1.44 | 2.60 | 10.40 |
| 28 | 0.96 | 0.77 | 3.08 | 1.31 | 1.88 | 7.52 |
| 29 | 1.08 | 1.05 | 4.20 | 1.25 | 1.60 | 6.40 |
| 30 | 0.88 | 0.63 | 2.52 | 1.40 | 2.35 | 9.40 |

Mean 3.52 6.58

the lung and kidney homogenates but not in the plasma. In the majority of the test samples (27), the level of kanamycin in the lung was found to be equal to or greater than that in the kidney.

The range of kanamycin levels in the lung homogenates was from 2.68 to 15.00 μg/g, with a mean value of 6.58 μg/g. The range in the kidney homogenates was from 1.64 to 5.48 μg/g, with a mean value of 3.52 μg/g.

DISCUSSION

The results of this study show that kanamycin, when administered to rats in aerosolized form, will be found in detectable levels in the tissues of the lungs and kidneys. Apparently the drug is
absorbed at the alveolar level. [Administration via the alimentary canal has been shown to yield minimal absorption in clinical study (2).] Further, the amount of the drug ingested by the test animals, via grooming activity, would be minimal.

The absence of kanamycin in the plasma and the presence of significant concentrations in the lung homogenates after 24 hr show that the drug is retained in pulmonary tissue.

LITERATURE CITED
1. Bilodeau, M., J. C. Roy, and M. Giroux. 1966. Studies of absorption of kanamycin by aerosolization. *Ann. N.Y. Acad. Sci.* 132:870-878.
2. Cohn, I., Jr. Kanamycin as an intestinal antiseptic and in the treatment of peritonitis: resume of clinical experience. *Ann. N.Y. Acad. Sci.* 132:860.
3. Grove, D. C., and W. A. Randall. 1955. Assay methods of antibiotics: a laboratory manual, p. 34. Medical Encyclopedia Inc., New York.
4. Prokhorova, I. I. 1968. Absorbability of kanamycin upon inhalation administration of the aerosol. *Antibiotiki* 13:351.