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Amantadine Small-Particle Aerosol: Generation and Delivery to Man (40551)

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The Collison nebulizer was developed in the early 1930s by W. E. Collison for use in inhalation therapy (1). Since then, it has been utilized in numerous experiments to deliver small particle aerosols (<5 μm) to the lower respiratory tract of both laboratory animals and man. These applications include aerosol vaccination and challenge experiments with live bacterial and viral agents (2, 3), as well as the delivery of antimicrobial and chemotherapeutic compounds directly to the lung (4, 5). The efficacy of aerosol therapy with antiviral compounds against influenza A infection in mice has been well established (6–9).

In one of several studies, Walker and his associates (9) gave amantadine small-particle aerosol 22 hr per day for 4 days beginning 72 hr after inoculation of mice with influenza A virus when virus titers in the lung were at their maximum ($\geq 10^7$ EID_{50} per lung). Results showed a high rate of survival in treated animals and a low rate of survival in controls. In parallel studies, daily intraperitoneal doses of amantadine in amounts much greater than those administered by aerosol showed no protective effect.

We found that 8-hr daily treatments with amantadine small-particle aerosol in mice was just as effective as aerosol given 23.5 hr daily (see Results). In these studies the concentration of amantadine in the aerosol was the same for both groups so that animals treated for 8 hr received only about one-third the dose given the 23.5-hr group.

On the basis of the favorable results in mice, studies were made of the absorption and tolerance of amantadine small-particle aerosol in normal volunteers. The results indicated the feasibility of this method of treatment of patients with influenza A infection. The present report describes the aerosol apparatus and its operating characteristics as developed for human use.

Materials and Methods. Aerosol system. The continuous aerosol therapy system was constructed at Johnson Spacecraft Center, Houston, Tex., and was modeled after a design developed at the U. S. Army Medical Research Institute of Infectious Diseases in Frederick, Md. (10). A schematic of the Baylor aerosol system is depicted in Fig. 1. The system can be operated with a portable air compressor, wall-outlet air from a central service compressor, or by air from a compressed air cylinder. Air enters the system and is regulated at 26 psi. It is then passed through a drying chamber to remove moisture and an "ultrapure" 0.3-μm filter. The dry (<15% r.h.) filtered air is then fed to two flow meters equipped with regulating valves. One flow meter monitors air going to the aerosol nebulizer, and the other controls the flow to the mixing chamber. The dry air lowers the relative humidity of the newly generated moisture-saturated aerosol and reduces the mass median diameter of the particles from about 4 to between 1 and 2 μm (>95% less than 5 μm). In the present studies air was fed to the aerosol generator at 7 to 8 liters/min and to the drying chamber at 4 to 5 liters/min, with a flow of about 12.5 liters/min to the face mask. A nipple on the outlet port of the mixing chamber serves as a receptacle for Smooth-Flo Corr-A-Tube II tubing. This tubing has an inner lining to reduce the impaction of aerosol particles on the flexible ribs in the tubing. A modified Ohio Medical Products Model 100, O_2 mask of the nonrebreathing type is used to deliver the aerosol to the subject. Only the face mask and breathing bag are utilized. An opening is made into the apex of the bag for insertion of the Smooth-Flo tubing, and the valves are replaced with American Optical Safety Division "Sure Guard" respiratory valves. These valves maintain sufficient back pressure to keep the 1-liter bag inflated with aerosol.

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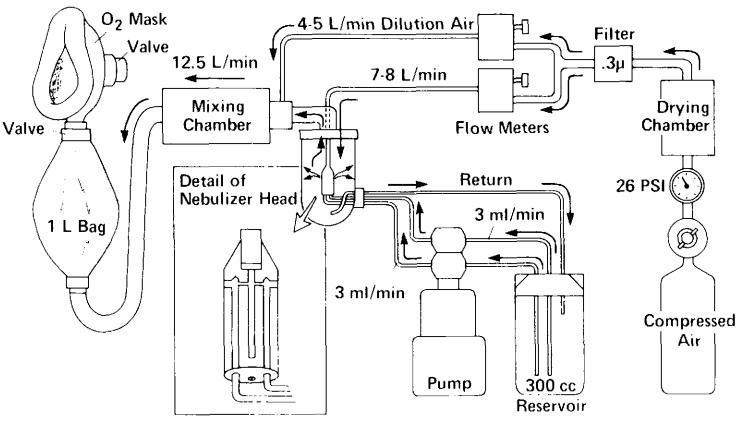

FIG. 1. Schematic of aerosol treatment apparatus for human use. (See text for description of operation.)

Generation of aerosol. Aerosol was generated in a Collison nebulizer made from polyvinyl chloride (PVC) according to the Ft. Detrick design. PVC is more easily machined and is cheaper than stainless steel used in earlier models. Amantadine solution was fed to two of three atomizer ports on the nebulizer head by peristaltic pumps (Fig. 1). The third port was sealed. Effluent liquid was recirculated to the liquid reservoir. It was found that a reservoir of 300 ml of amantadine solution was large enough to control adequately the concentrating effect of excess evaporation of water during atomization of aerosol for an 8- to 10-hr period of operation (see Results).

Uranine. Uranine dye (Fisher Scientific Co., Fair Lawn, N.J.) dissolved in distilled water (1.0% solution) was used to characterize the aerosol generators.

Amantadine hydrochloride. Amantadine hydrochloride Lot No. 77041 was provided by Endo Laboratories, Garden City, N.Y. It was prepared sterile for human use in distilled water, 15 mg/ml, in rubber-stoppered 100-ml bottles by the pharmacy of The Methodist Hospital, Houston, Tex.

Aerosol sampling. The all-glass impinger (AGI) was used to collect aerosol for assay of amantadine and uranine (11). Triplicate samples were taken for uranine, and duplicate samples were taken for amantadine assays. Amantadine was assayed by a gas chromatographic method devised in this laboratory. Uranine was assayed fluorometrically.

Particle sizes were determined with the Andersen sampler (12). Uranine aerosol was collected in 2% Noble agar. It was equilibrated overnight with a measured volume of distilled water which was assayed for uranine. Control studies showed the validity of the assay. Amantadine was similarly assayed in 12% gelatin. The gelatin was melted at 37°; and after thorough mixing, samples were diluted 1:4 in distilled water.

Experimental infection of mice. Details of this study are described in the legend of Fig. 2.

Results. Aerosol treatment of experimental influenza A infection in mice. Amantadine aerosol was administered using the aerosol generator described in this report with the exception that the face mask was omitted, and the tubing from the mixing chamber was attached to plastic mouse cages with sealed-on plastic tops as described in the report by Young *et al.* (10). Figure 2 shows that the mortality in both groups of treated mice was 25% compared to 66% among controls. The difference in survival rates between treated and untreated mice was highly significant ($P < 0.00007$, Wilcoxon rank sum test). Note that the concentration of amantadine in the liquid reservoir was the same for the mice as that used for human studies (15 mg/ml). Mice inhaled about 25 ml/min of an aerosol containing 60 μg/liter (13). With an estimated 30% retention of inhaled drug, a 25-g mouse would receive 8.6 mg/kg/8-hr treatment (13). On a similar basis a 60-kg man inhaling 8 liters/min would receive 2.03 mg/kg/8-hr day of amantadine (14).

Operating characteristics of the aerosol generator. To evaluate the operation of the aerosol apparatus, measurements were made of concentrations of amantadine in reservoir

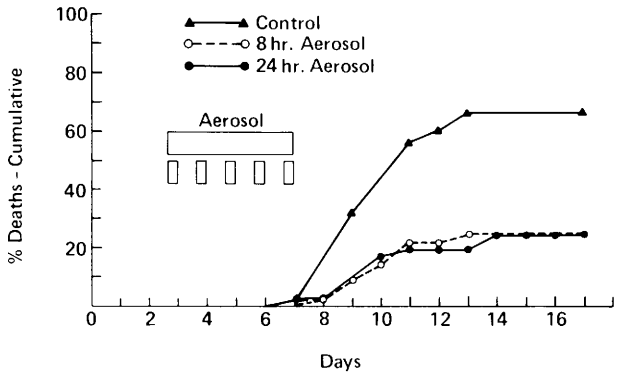

FIG. 2. White Swiss mice about 7 weeks of age were exposed to a small-particle aerosol of mouse-adapted influenza A/Aichi/1968 (H3N2), 1:100 dilution of allantoic fluid pool (titer 1.6×10^6/ml) for 15 min (30 EID_{50}, approximately 5 LD_{50}). Treatment with amantadine, 15 mg/ml in distilled water in the reservoir was begun 64 hr after inoculation. One group was treated 8 hr daily for 5 days; another was treated 23.5 hr a day for 4.3 days. The aerosol contained 60 mcg per liter of amantadine. 8-hr group: $n = 40$, 23.5-hr group: $n = 40$, control group: $n = 49$. (Wilcoxon rank sum test: $Z = 3.8$ in 8- and 23.5-hr groups vs controls, $P < 0.00007$).

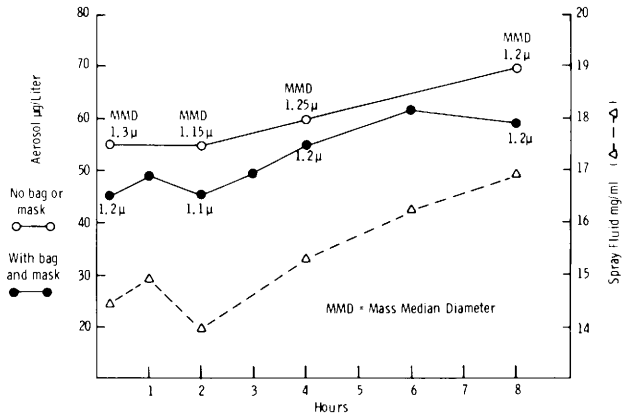

FIG. 3. Concentration of amantadine in liquid reservoir and in aerosol with and without face mask and bag during 8 hr of continuous operation. Aerosol concentrations are indicated at left side of diagram. Amantadine concentration in liquid reservoir (spray fluid) is shown at right side of diagram. MMD = mass median diameter.

fluid, aerosol from the mask, and aerosol without the mask at intervals during an 8-hr period of continuous operation (Fig. 3). Estimates indicated that the approximate daily recommended oral dose of 200 mg could be administered by aerosol in this period.

During the 8 hr of operation the concentration of drug in the reservoir gradually increased and was 17% higher than the initial concentration after 8 hr. There was a similar increase in concentration of amantadine in aerosol during this period, approximately 30%, with or without the mask. The mass median diameter (MMD) remained nearly constant throughout (about 1.2 μm). The reservoir contained 300 ml of amantadine solution, 15 mg/ml, in this study. The difference in concentration of amantadine in aerosol with and without the mask, approximately 11%, was due to deposition of drug on the face mask, which became visible as a white powder after a short period of operation. When 200 ml was contained in the reservoir, the concentration increase was greater (Table I); however, in this circumstance as well, the particle size remained nearly constant at 1.2 μm MMD.

Comparison of polyvinyl chloride (PVC) and stainless-steel nebulizer heads. As can be seen in Table II, the PVC nebulizers proved to be efficient aerosol generators. No significant differences were noted between the PVC neb-

TABLE I. CONCENTRATION EFFECT DUE TO WATER LOSS FROM 200- and 300-cc VOLUMES OF AMANTADINE SOLUTION AFTER 4 hr OF OPERATION

	At start: Amantadine in reservoir (mg/ml)	Aerosol (μg/liter)	MMD (μm)	After 4 hr: Amantadine in Reservoir (mg/ml)	Percentage increase	Aerosol (μg/liter)	Percentage increase	MMD (μm)
200 cc (without mask)	14.2	63.6	1.2	17.0	19.7	79.7	25.3	1.3
300 cc (without mask)	14.5	55.5	1.3	15.3	5.5	60.4	8.8	1.2

TABLE II. EVALUATION OF NEBULIZER HEAD WITH URANINE DYE

	Mass median diameter (μm)	Aerosol concentration (μg/liter)
Stainless steel[a]	1.55	48.75
PVC No. 1	1.52	48.8
PVC No. 2	1.50	47.3

[a] Loaned by U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Md.

ulizers and the standard stainless-steel head ($P > 0.6$).

Discussion. This report has described an aerosol generator and its operating characteristics for use in viral chemotherapy. Studies of the absorption and tolerance in normal volunteers of an aerosol of amantadine produced with this equipment have indicated rapid and substantial absorption of drug and an acceptable level of tolerance. In addition, in a preliminary clinical study of influenza A infection, amantadine aerosol given 10 hr daily for 3 days was associated with rapid recovery and was well tolerated.

The device can deliver precise dosages of drug in aerosol; and with knowledge of the minute volume of expired and an estimated percentage deposition of inspired aerosol, reasonably accurate predictions of the amount of retained drug can be made. The literature is deficient in studies of the degree of retention of inhaled particles, and such studies will be undertaken in this laboratory.

Using the somewhat arbitrary criteria we have for retention of aerosol in mice and man, the estimated dosages in mice were approximately fourfold those in man when similar exposure periods were employed and the amantadine concentration in the spray fluid was 15 mg/ml. Whether the smaller retention will diminish the potential for effective aerosol treatment in man will be determined by studies currently in progress. The dosage in man can probably be safely increased, if necessary, by prolonging the treatment period or by increasing the concentration of amantadine in the spray fluid.

Summary. An aerosol generator suitable for administration of a small-particle aerosol of amantadine for treatment of influenza A infection in man is described. As currently operated, the usual daily recommended oral dose of amantadine of 120 to 200 mg can be given in 8 to 10 hr of inhalation of the aerosol. Limited clinical study indicates the safety and probable efficacy of this method of treatment.

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