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Amantadine Aerosols in Normal Volunteers: Pharmacology and Safety Testing

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The acceptability and pharmacology of intermittent aerosol administration of amantadine was assessed in healthy volunteers. Amantadine solutions of 2.5, 1.5, or 1.0 g/100 ml were used for 12 30-min, twice-daily aerosol treatments in 15 subjects. Overall, the aerosol treatments were well tolerated. During and up to 1 h after aerosol exposures, nasal irritation, rhinorrhea, dysgeusia, or a combination of symptoms was experienced by some of the subjects receiving either of the two higher amantadine concentrations. Aerosol treatments were associated with small but statistically significant decreases in maximal expiratory flow rates. One hour after aerosol treatments with the 1.0-g/100 ml solution, amantadine levels in nasal wash samples (mean, 30.3 μg/ml; range, 1.7 to 108 μg/ml) greatly exceeded blood and nasal wash levels reported after oral administration. Amantadine can be administered safely by small-particle aerosol to humans in doses that could be expected to exert an antiviral effect in influenza A virus infections.

Despite available immuno- and chemoprophylaxis, influenza A virus infections continue to cause considerable morbidity and mortality, especially among the very young, the elderly, and those with chronic illness. The increased morbidity and mortality in these groups is mainly due to pulmonary complications (10, 24). Although less frequent than secondary bacterial pneumonia, primary influenza A virus pneumonia continues to cause high mortality and chronic pulmonary dysfunction in survivors (41).

Amantadine hydrochloride is licensed by the U.S. Food and Drug Administration for use in the prevention and symptomatic management of respiratory tract infections caused by influenza A virus. Double-blind, controlled clinical studies of orally administered amantadine have demonstrated a therapeutic effect in nonpneumonic natural influenza affecting previously healthy adults. Amantadine administration early in the course of illness has resulted in more rapid resolution of fever, clinical signs and symptoms (36, 40), frequency and magnitude of virus shedding (19; L. P. Van Voris, F. G. Hayden, R. F. Betts, R. G. Douglas, Jr., and W. A. Christmas, Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., abstr. no. 483, 1978), and pulmonary function abnormalities (22) as compared with placebo. However, even in uncomplicated influenza, the effects of oral amantadine are not striking, and its role in the management of more severe illness, such as primary influenza A virus pneumonia, remains undefined. In addition, increases in oral dosage beyond those currently utilized are often associated with unacceptable central nervous system side effects (3, 29).

Studies of experimentally induced influenza A virus infections of animals have found greater therapeutic effects when amantadine was administered by the respiratory route as a small-particle aerosol, as compared with parenteral (38) or oral (7, 35) administration. These advantages of topical therapy were demonstrated with dosages of amantadine lower than those given by the other routes.

The present study was undertaken in healthy volunteers to determine the acceptability, safety, and certain pharmacological aspects of amantadine hydrochloride delivery to the respiratory tract in small-particle aerosol form. The results indicate that amantadine hydrochloride can be administered safely by intermittent aerosol, and that after aerosol administration amantadine concentrations in the upper respiratory secretions greatly exceed blood and nasal wash levels found after oral administration.

MATERIALS AND METHODS

Volunteers. Fifteen healthy adults, aged 18 to 35 years, participated in the study. Before, and after the

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study, history and physical examinations, complete blood counts, blood chemistries, and urinalysis were performed in each. The five female subjects had a reliable history of contraception and negative pregnancy tests. Smokers were included. All subjects were informed of the objectives, procedures, and hazards of the study, and all gave written informed consent.

Drug administration and study design. Amantadine hydrochloride was supplied as crystalline powder by Endo Laboratories, E. I. Dupont de Nemours & Co., Wilmington, Del. Aerosol solutions were prepared by the Strong Memorial Hospital Pharmacy by dissolving amantadine in sterile, distilled water. The pH of the solutions was adjusted to between 6.0 and 6.5 with sodium hydroxide, and the solutions were filtered into sterile, rubber-stoppered bottles. Groups of five volunteers each received aerosol treatments with one of three amantadine solutions: group I, 2.5 g/100 ml; group II, 1.5 g/100 ml; and group III, 1.0 g/100 ml. The aerosol mist was generated with Devilbis no. 40 glass nebulizer (Devilbis Co., Somerset, Pa.). The vent was plugged, and compressed laboratory air was delivered through the base at a constant air flow rate of 15 to 16 liters/min. The solution utilization rate was 0.3 ml/min. This delivery system produces a polydisperse aerosol mist with an aerodynamic mass median diameter of approximately 3 μm (20, 26). The aerosol mist was directed to the face of each subject with a large-bore, flexible plastic tube which served as a reservoir and a face mask. Subjects were instructed to breath tidally through both mouth and nose. Thirty-minute aerosol treatments were administered at 8 a.m. on day 1 and twice daily at 8 a.m. and 4 p.m. thereafter, until a total of 12 treatments was completed.

Pharmacological studies. The extent of systemic drug absorption was assessed by measurement of amantadine content in the urine. Twenty-four-hour urine specimens and three blood samples at hourly intervals were collected after completion of the first aerosol treatment. In healthy adults, approximately 56% of a single oral dose is excreted in a subsequent 24-h urine collection (1). The average systemically absorbed dose for each group was calculated by dividing the mean 24-h urinary content by this correction factor of 0.56. The amount of amantadine retained in the respiratory tract during each aerosol treatment could not be measured directly. The retained dose was estimated as follows. Estimated retained dose = concentration of amantadine in aerosol (micrograms per liter aerosol) × minute ventilation (liters per minute) × duration of exposure (minutes) × retention rate. The concentration of amantadine in the aerosol mist was derived from the known output characteristics of the nebulizer (26). The retention rate was assumed to be 0.5 (J. S. Walker, personal communication), although this may be an underestimate (13). The minute ventilations were taken from standards corrected for age and height of the subjects (31). Based on these calculations, the mean retained amantadine doses per 30-min exposure were estimated to be 35, 20, and 15 mg for subjects in groups I, II, and III, respectively. Nasal washes with lactated Ringers solution (5 ml into each nostril) were obtained less than 10 min after and at 60 min after completion of an aerosol treatment in subjects in group III. These collections were not done sequentially after the same aerosol exposure and were not obtained concurrently with blood samples.

Amantadine hydrochloride concentrations in blood, urine, and nasal wash samples were determined on coded samples with a gas-liquid-chromatographic assay (1) at the Stine Laboratory, E. I. Dupont de Nemours & Co.

Toxicity monitoring: (i) clinical evaluations. Each subject was examined daily shortly after completion of the morning aerosol treatment. Each was questioned with regard to symptoms of nasal irritation or obstruction, sneezing, sore throat, cough, wheezing, headache, lightheadedness, anorexia or nausea, and tearing or eye discomfort. Specific signs of nasal discharge, mucus membrane edema or erythema, and wheezing or ronchi were sought.

Complete blood counts, blood chemistries, and urinalysis were performed on admission to the study, on days 3 and 7 of aerosol amantadine treatments, and 1 week later. The blood chemistries included total protein, albumin, calcium, inorganic phosphorus, cholesterol, blood urea nitrogen, uric acid, creatinine, total bilirubin, alkaline phosphotase, lactate dehydrogenase, and alanine aminotransferase.

(ii) Pulmonary function tests. Noninvasive pulmonary function tests were performed before and after a distilled water aerosol treatment, on days 3 and 6 of amantadine aerosol treatments, and at 2 to 4 weeks after completion of aerosol treatments. To maximize detection of abnormalities, these tests were performed within 15 min of the end of aerosol exposure. Maximal expiratory flow-volume curves were obtained by previously described methods (23) with the subjects breathing air, and they were repeated after wash out of the lungs with an 80% helium–20% oxygen mixture. Data were expressed as maximal flow ($\dot{V}_{max}$) in liters per second, divided by the vital capacity of each subject to produce a value in vital capacity per second ($\dot{V}_{max}$/vital capacity = vital capacity/second) to correct for variations in lung size (16). Total pulmonary resistance (R_T) was measured at 3 cycles per second with the forced oscillometric technique by previously described methods (8, 22). The mean of four determinations obtained at functional residual capacity during normal breathing was recorded. The subjects then inhaled five tidal breaths of 1.6% histamine diphosphate solution (5) delivered by a Devilbis no. 42 nebulizer (Devilbis Co., Somerset, Pa.) at an airflow of 6 liters/min. Measurements of R_T were then repeated. Airway reactivity was expressed as the numerical difference between R_T before and after histamine challenge (ΔR_T).

Microbiological methods. Since intercurrent respiratory infections could adversely affect the results of the clinical evaluations and pulmonary function tests used in toxicity monitoring, subjects were screened for the presence of respiratory tract pathogens. Throat and nasopharyngeal swabs for virus (21) and throat swabs for mycoplasma (18) and group A, beta-hemolytic streptococci (6) isolation were performed on the same days as pulmonary function tests. Cell cultures of human embryonic lung (WI38), primary rhesus monkey kidney, and human epidermoid carcinoma (Hep-2) were used for virus isolation.

Statistical methods. With each subject serving as

his or her own control, pulmonary function data were analyzed for significance by both conventional parametric analysis (paired t test) and nonparametric analysis (Wilcoxon rank series analysis) (14, 33). In comparing sequential changes in a group, only P values ≤ 0.05 by both methods of analysis were considered significant.

RESULTS

Pharmacological data: (i) plasma levels. Low plasma concentrations of amantadine were present at 1 h after completion of the aerosol treatments (Table 1). Amantadine remained detectable in plasma at 3 h after aerosol exposure. The timing of the peak concentration varied among individuals, but usually occurred at 120 or 180 min after completion of the aerosol. The mean peak blood level was significantly lower in group III as compared with that in group II ($P < 0.05$, Student's t test), but was quite low in both groups. These groups were otherwise similar with respect to age, weight, height, and initial spirometric measurements.

Three subjects in group I had plasma amantadine determinations at 1 h after the twelfth aerosol treatment to assess possible drug accumulation. These levels (0.05, 0.06, and 0.06 μg/ml) were similar to those obtained at 1 h after the initial aerosol exposure (0.04, 0.07, and 0.02 μg/ml), and they did not indicate significant accumulation.

(ii) Urinary excretion. Twenty-four-hour urine collections after a single, 30-min aerosol exposure contained considerable quantities of amantadine (Table 1). These urine collections contained up to 38% of an estimated retained dose and reflected considerable, though variable, systemic absorption of the drug. Based on previous studies which have shown that approximately 56% of a single oral dose is excreted in a subsequent twenty-four-hour urine collection (1), the calculated, average systemically absorbed doses were 24.2, 9.1, and 9.5 mg per aerosol treatment in groups I, II, and III, respectively. The ratio of the urinary drug excretion between groups was approximately proportional to the ratio of amantadine concentration in the aerosol solutions.

(iii) Nasal wash levels. The nasal wash content of amantadine was determined only for group III. The average amantadine concentration just after completion of the aerosol treatments was 111.3 μg/ml of nasal wash (range, 25 to 283.5 μg/ml). At 60 min after completion of the aerosol, the average amantadine concentration was 30.3 μg/ml of nasal wash (range, 1.7 to 108 μg/ml). Because collection of multiple nasal washings in the same individual after a single aerosol exposure could interfere with recovery of amantadine, the rate of drug clearance from nasal secretions could not be directly measured. The mean half-life of disappearance of amantadine from nasal secretions appeared to be about 30 min. When corrected for a minimum dilution factor of at least threefold due to the nasal wash procedure (34), mean 1-h levels (91 μg/ml) exceeded mean peak plasma concentrations (0.02 μg/ml) by greater than 4,000 fold.

Tolerance: (i) clinical evaluations. No subject developed lower respiratory tract signs or symptoms. Although evidence of nasal irritation was apparent at higher amantadine concentrations, no local adverse effects were observed at the 1.0-g/100 ml concentration (group III). All five subjects receiving the 2.5-g/100 ml concentration noted minimal to moderate burning discomfort of anterior nares, rhinorrhea, and unpleasant taste during and up to 1 h after aerosol exposure. Three of five had hyperemia of the anterior nasal mucosal. Four of five subjects receiving the 1.5-g/100 ml concentration noted minimal local nasal irritation and slight rhinorrhea during the first one to four treatments. Symptoms resolved in three of the four during subsequent aerosol exposures. Subjects in group III did not experience any noteworthy upper-respiratory tract signs or symptoms.

One subject in group II was removed from the

TABLE 1. *Plasma and urinary concentrations of amantadine hydrochloride after single aerosol treatment*

Amantadine concn in aerosol solution (g/100 ml)	Amantadine concn in plasma (μg/ml) at:[a]			Peak (μg/ml)[b]	Urine excretion (mg/24-h collection)[a]
	60 min	120 min	180 min		
2.5	0.04 (0.02–0.08)	0.04 (0.02–0.07)	0.02[c]	—[c]	13.5 (6.8–23.6)
1.5	0.03 (0.02–0.07)	0.10 (0.04–0.17)	0.07 (0.03–0.17	0.10 (0.03)	5.1 (ND–10.7)[d]
1.0	(ND–0.01)	0.02 (ND–0.04)	0.01 (ND–0.03)	0.02 (0.01)	5.3 (3.4–6.2)

[a] Each value represents mean of a group of five subjects; values in parentheses indicate range.

[b] Each value represents mean ± standard error of the highest observed amantadine concentration for each subject in the group.

[c] Only one subject was tested at 180 min

[d] ND, No amantadine detectable.

protocol because of the sudden onset of sneezing and marked rhinorrhea approximately 30 min after completion of her seventh aerosol treatment. Her symptoms abated over the next 48 h. She experienced no lower-respiratory or constitutional symptoms, and pulmonary function tests showed no change. Wright-stained smear of nasopharyngeal secretions showed no eosinophils. Aerosol rechallenge with an amantadine concentration of 0.5 g/100 ml 3 weeks after this episode did not provoke symptoms. For all subjects serial hematological, blood chemistry, and urinalysis studies showed no remarkable changes. One subject developed an isolated, asymptomatic bilirubin elevation to 1.4 mg% at the time of follow-up testing. This value had decreased to 0.4 mg% 1 week later.

(ii) Pulmonary function tests. The results of serial pulmonary function tests for 14 of the 15 subjects tested are given in Table 2. The subject in group II who was dropped from the protocol is not included. A single, 30-min distilled water aerosol exposure was associated with a significant decrease in maximal expiratory flow rates while the subjects were breathing an 80% helium–20% oxygen mixture. Repeated aerosol amantadine treatments did not cause any significant changes in measurements of forced expiratory volumes, total pulmonary resistance, or the change in pulmonary resistance after an inhalation of histamine. Repeated aerosol treatments were associated with small but statistically significant decreases in maximal expiratory flow rates on days 3 and 7 of the study compared with base-line measurements. These values were also significantly less than those seen after a single distilled-water aerosol. These values returned toward base line ones after discontinuation of aerosol exposures. These changes did not appear to relate to the concentration of amantadine in the aerosol solution, but the number of subjects in each group was small. In general, those subjects demonstrating the greatest decreases in forced flow rates after amantadine aerosol were the same subjects manifesting the greatest response to water aerosol. For example, the change in V_{max50} air after amantadine aerosol on Day 3 correlated with effect of water aerosol noted at base-line ($r = 0.61$ $P < 0.01$). The effect of repeated distilled-water aerosol treatments was not determined. Intercurrent respiratory tract infections were not detected clinically or by repeated viral, mycoplasma, or bacterial throat cultures.

DISCUSSION

This study evaluated the feasibility of administering amantadine hydrochloride by small-particle aerosol to human subjects. Topical application of antimicrobial agents to the respiratory tract attempts to provide local antimicrobial activity greater than that achievable by other routes of administration and concomitantly to minimize the risk of systemic drug toxicity (27). On the basis of in vitro susceptibility testing, oral administration of amantadine yields blood and respiratory secretion levels that would be expected to possess marginal antiviral activity against influenza A viruses. Depending on the assay system and strain of influenza A virus, in vitro antiviral activity has been observed at

TABLE 2. *Pulmonary function studies in 14 subjects receiving aerosol amantadine treatment*[a]

Time of measurement	FVC[b] (ml)	FEV_1[c] (ml)	V_{max50} Air[d]	V_{max50} He[e]	R_T[f]	ΔR_T[g]
Base line	4,475 (956)	3,840 (823)	1.12 (0.43)	1.61 (0.67)	2.96 (0.62)	1.04 (0.84)
After water aerosol	4,546 (936)	3,990 (1,055)	1.11 (0.40)	1.54 (0.58)[h]	3.01 (0.61)	0.93 (0.47)
Amantadine day 3	4,528 (875)	3,795 (728)	1.01 (0.38)[i]	1.49 (0.68)[j]	2.82 (0.65)	0.95 (0.59)
Amantadine day 6	4,463 (868)	3,692 (703)	0.99 (0.41)[k]	1.39 (0.62)[l]	2.87 (0.56)	0.95 (0.67)
Follow-up	4,423 (896)	3,733 (708)	1.07 (0.39)	1.49 (0.60)[m]	3.02 (0.71)	0.73 (0.52)

[a] Results expressed as mean ± standard deviation for 14 subjects.
[b] Forced vital capacity (milliliters).
[c] Forced vital capacity in 1 s (milliliters).
[d] Maximal expiratory flow rate at 50% of vital capacity with subject breathing room air.
[e] Maximal expiratory flow rate at 50% of vital capacity with subject breathing an 80% helium–20% oxygen mixture.
[f] Total pulmonary resistance (centimeters of water per liter per second) measured by oscillometric technique.
[g] Numerical difference in R_T (centimeters of water per liter per second) measured after and before histamine aerosol.
[h] $P < 0.02$ versus base line (Wilcoxon signed-ranks test).
[i] $P < 0.01$ versus base line; $P < 0.05$ versus after water.
[j] $P < 0.01$ versus base line.
[k] $P < 0.01$ versus base line; $P < 0.02$ versus after water.
[l] $P < 0.01$ versus base line; $P < 0.01$ versus after water.
[m] $P < 0.05$ versus base line.

amantadine concentrations ranging from <1 to 25 μg/ml (4, 15, 32). The median effective doses for suppression of hemagglutinin production in Rhesus monkey kidney cell culture ranged from 3.1 to 7.5 μg/ml for a variety of influenza A viruses (12). In the same study, the A/New Jersey/8/76 strain was inhibited by concentrations less than 1.0 μg/ml. With a plaque inhibition assay, the median inhibitory concentration of amantadine was 0.3 to 0.4 μg/ml for contemporary influenza A viruses, including strains of the H3N2, HSW1N1, and H1N1 subtypes (F. G. Hayden and R. G. Douglas, Jr., unpublished data).

In humans, amantadine is well absorbed after oral administration, with peak blood levels of 0.6 μg/ml after a 5-mg/kg dose (1). With prolonged oral administration of 200 mg/day, steady-state blood levels remain less than 1.0 μg/ml (11) if renal function is normal (17). Amantadine levels in respiratory secretions appear to approach plasma concentrations after oral administration (34). During repeated administration of amantadine (100 mg every 12 h to seven volunteers), pooled nasal wash samples were taken from 2 h before and 2 h after an oral dose (34). These samples had an average amantadine concentration of 0.07 μg/ml (range, 0.02 to 0.20 μg/ml).

In contrast, aerosol administration of amantadine yielded concentrations in the upper respiratory tract secretions that greatly exceed blood and nasal wash levels found after oral administration. In the present study, the mean amantadine concentration observed in nasal wash specimens collected 1 h after the completion of a 30-min aerosol exposure was over 30 μg/ml, or several hundred-fold higher than those observed after oral administration (34). Furthermore, the upper respiratory tract levels of amantadine after aerosol administration surpassed those which have been shown to be inhibitory for influenza A viruses in vitro (4, 12, 15, 32). However, the magnitude and duration of drug levels in respiratory tissues are probably critical determinants of antiviral activity, and tissue levels were not directly measured in the present study.

Dose-related side effects, particularly those referrable to the central nervous system, limit the amount of amantadine that can be orally administered. Aerosol delivery of amantadine reduces this problem, because the drug is delivered gradually. Amantadine is highly water soluble (15), and aerosol delivery is followed by rapid appearance of the drug in the urine (V. Knight, K. Wilson, S. Wilson, K. Bloom, and P. M. Stevens, Clin. Res. **26**:800A, 1978). This presumably indicates that topically administered amantadine is readily absorbed across the respiratory membrane, although gastrointestinal absorption of the swallowed drug is also a possibility. In the present study, the extremely low plasma levels observed after aerosol administration reflect that the retained doses were only a fraction of the usual 200-mg/day oral dose. Since increasing the duration of aerosol exposure would directly increase the total retained dose of amantadine, higher total doses could be administered with prolonged or continuous aerosol exposure.

In accord with previous studies of aerosol amantadine therapy in animal models of influenza (7, 35, 38), aerosol treatments were generally well tolerated. Nasal irritation, rhinorrhea, and disagreeable taste occurred in all subjects receiving treatments with a 2.5-g/100 ml solution concentration, but no adverse effects were observed with the 1.0-g/100 ml concentration. A 4.0-g/100 ml solution of a related compound, cyclooctylamine hydrochloride, has also been associated with transient nasopharyngeal irritation upon intranasal administration (37). An amantadine solution of 1.5 g/100 ml did not cause local adverse effects during prolonged aerosol treatments of seven volunteer subjects (V. Knight et al., Clin. Res. **26**:800A, 1978). Solution concentrations in the 1.0 to 1.5-g/100 ml range would be appropriate for future clinical studies involving aerosol or topical administration of amantadine.

Several other factors may have contributed to the occurrence of local side effects in this study. The aerosol delivery system used a high air flow rate which was directed toward the face of the subject. In addition, during operation of air blast nebulizers, such as the Devilbis no. 40, evaporation of the solvent causes a continuous increase in the solute concentration in the remaining liquid (26). We sought to minimize this problem by frequently replenishing the reservoir of the nebulizer with fresh solution during operation. Other delivery systems which incorporate large volume reservoirs offer an alternative approach.

Although most patients with influenza do not have clinically detectable pneumonia, pulmonary function abnormalities frequently accompany even nonpneumonic influenza infection (22, 23). In this setting, the possible adverse effects of aerosol therapy are important considerations. Inhalation of chemical irritants and nonspecific irritants, including water mist, can provoke bronchoconstriction and increased resistance to airflow in subjects without bronchopulmonary disease (2, 25, 28, 39). Patients with underlying obstructive airways disease may experience increased airway resistance, decreased flow rates, and decreased arterial oxygen ten-

sions when exposed to ultrasonic aerosols or high humidity (9, 30). Symptomatic airway obstruction has occurred in two subjects, one of whom had known asthma, after prolonged inhalation of an amantadine aerosol (V. Knight et al., Clin. Res. **26**:800A, 1978).

In the present study none of the subjects developed clinical evidence of airways obstruction. Serial pulmonary function tests performed within 15 min after the completion of aerosol exposure found no significant changes in forced expiratory volumes or in tests selected to assess bronchial reactivity (5). Repeated aerosol amantadine treatments were associated with minor but statistically significant decreases in maximal expiratory flow rates. These changes were of small magnitude and resolved after discontinuation of aerosol treatments. As anticipated from the particle size distribution of the aerosol mist (13, 20, 26), the changes offered indirect evidence that aerosol was being deposited in the lower respiratory tract. The clinical and physiological importance of these changes were considered to be minimal in relation to healthy subjects, but potentially of greater importance in patients with preexisting lung disease. We did not determine whether these pulmonary function changes were associated with amantadine treatments specifically or were possibly related to repeated aerosol exposures in general.

Oral administration of amantadine in nonpneumonic influenza is associated with a more rapid resolution of abnormalities in peripheral airway function compared with that of placebo (22, 23). The mechanism of its beneficial effect is postulated to relate to its antiviral activity and reduction of the extent of epithelial damage. Orally administered amantadine does not result in a direct bronchodilator or anticholinergic effect (23). The present study confirms that topical administration of amantadine to the respiratory tract does not cause bronchodilation or attenuate the airway response to an histamine inhalation.

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