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COMPARATIVE THERAPEUTIC EFFECT OF AEROSOLIZED AND ORAL RIMANTADINE HCl IN EXPERIMENTAL HUMAN INFLUENZA A VIRUS INFECTION

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Thirty-six adult volunteers were inoculated intranasally with 7.2 $\log_{10}$ egg infectious doses 50% of an attenuated A/Khabarovsk/77/H1N1 virus. Twenty-four hours later volunteers were begun on both aerosol treatments (rimantadine HCl 25 mg in saline or saline, 10 min exposure twice daily) and oral medications (rimantadine HCl 50 mg or placebo every 6 h, three times daily) which were administered for 5 days. Virus-positive volunteers receiving placebo by both of the two routes had a peak in clinical illness scores on the second treatment day (mean score 5.3), which was not observed in either the aerosol rimantadine (0.6) or oral rimantadine (0.9) treated volunteers. On the second treatment day, the proportion of virus-positive volunteers with elevated axillary temperature measurements and the mean peak temperature measurement were also significantly reduced in both drug groups. No significant effects on the duration of virus shedding were noted.

In experimental influenza A virus infection, characterized by mild clinical illness and short duration of virus shedding, low doses of aerosolized rimantadine had a therapeutic effect comparable to that found with larger doses of oral rimantadine.

influenza aerosol rimantadine

INTRODUCTION

Aerosol administration of antiviral drugs for the treatment of respiratory viral infections has received increasing attention. In both murine [8,11] and ferret [2] models of influenza A infection, aerosol delivery of amantadine has been shown to be therapeutically superior to oral [2,8] or parenteral [11] administration. The closely related drug, rimantadine HCl, has similarly been found to be highly protective against experimental influenza A virus infection in mice [9,11]. In human studies, both of these drugs, when given orally in doses of 200 mg/day to patients with uncomplicated influenza, have been shown to decrease the duration of fever, symptoms, and virus shedding [10]. Hayden and coworkers found that intermittent inhalations of small particle aerosols of amantadine HCl were associated with significantly faster resolution of both respiratory and systemic

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symptoms and a trend toward decreased quantities of virus shed in A/USSR/77/H1N1-infected students, as compared to placebo inhalations [3].

The purpose of the current study was to determine the therapeutic and antiviral activity of aerosolized rimantadine HCl as compared to placebo or orally administered rimantadine HCl in experimentally induced human influenza A virus infection.

PATIENTS AND METHODS

Patients

Thirty-six healthy, young adult volunteers of either sex were enrolled in the study. All had normal clinical examinations by an internist, otolaryngologist, and neurologist, as well as normal hematologic, electrocardiographic, chest radiographic and spirometric data. During the 4 days preceding and for one week after virus inoculation, subjects were housed in isolation quarters of the Clinical Center of the All-Union Research Institute for Influenza, Leningrad. Volunteers were told that they would receive both live influenza virus vaccinations which might cause symptoms and anti-influenza medications.

Complete blood counts, differential leukocyte counts, levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, blood urea, and conventional spirometry were measured prior to virus challenge, on the 4th day of drug therapy, and 1 day after completion of drug administration.

Virus

A clinical isolate of A/Khabarovsk/77/H1N1, which had been attenuated by 10 passages in embryonated hens' eggs, was used as the challenge virus. All subjects were inoculated with $10^{7.2}$ egg infectious doses 50% given by intranasal spray in a total volume of 0.5 ml.

Treatments

Volunteers were stratified on the basis of their baseline hemagglutination-inhibition (HAI) antibody to a challenge virus and then randomized into one of the three treatment groups. Twenty-four hours after virus inoculation, all volunteers were begun on both inhalation treatments and oral medications for the next 5 days. The aerosol treatment group received 10 min inhalations of rimantadine twice daily at intervals of 10–12 h and oral placebo tablets three times a day. The oral treatment group received rimantadine 50 mg three times a day at 6 h intervals and placebo aerosol treatments twice daily. The control group received placebo by both routes. The placebo tablets were similar but not identical to the rimantadine tablets in size, color, and shape.

The aerosol mist was generated by an ultrasonic nebulizer (TUR-URI-3, Dresden, G.D.R.). For inhalation treatments, 25 mg of rimantadine was dissolved in 3 ml of

normal saline; saline alone was used as the placebo solution. The mist was delivered to the subjects by means of a flexible plastic tubing and a tight fitting aviator mask. The volunteers were instructed to inhale with tidal breaths through their nose and exhale through their mouth.

Clinical evaluations

Volunteers were examined daily by study physicians unaware of the type of treatment administered. Specific symptoms and physical findings of constitutional (headache, feverishness, chills/sweats, myalgia, muscle tenderness, malaise, lethargy, anorexia/nausea, diarrhea) and respiratory (nasal obstruction, nasal discharge, sneezing, sore throat, hoarseness, pharyngitis, cough sputum production, tracheal tenderness) illness were rated in a semiquantitative manner from absent (0) to severe (+3). An individual illness score for each day and for the 5 day period after virus inoculation was generated by totaling the score for individual symptoms on these days.

Axillary temperature measurements were taken four times per day. Based on previous studies in this laboratory, recordings greater than or equal to 37°C were considered to be elevated.

The volunteers were questioned on the last two treatment days with regard to any untoward symptoms (nasal irritation, bitter taste, altered smell or taste, cough) occurring during or just after aerosol treatments.

Viral studies

Each morning before aerosol treatments, nasal secretions were collected for virus isolation in embryonated hens' eggs. Two blind passages were performed, before the culture was considered negative.

RESULTS

Infection

Baseline serum HAI antobody titers $\leqslant 1 : 8$ were present in nine volunteers in the aerosol treatment group, nine in the oral treatment group, and eight in the placebo group. The remaining subjects in each group had antibody titers ranging from 1 : 16 to 1 : 64. Twenty-three (64%) volunteers had virus isolated from nasopharyngeal secretions before the initiation of drug treatments at 24 h after virus challenge. The portion of virus-positive subjects constituted approximately two-thirds of each group (Table 1). Of those volunteers shedding virus at 24 h after inoculation, two out of eight treated with aerosol rimantadine, three out of eight with oral rimantadine, as compared to five out of seven placebo recipients (P=0.20 vs. aerosol, Fisher's exact test) continued to shed virus at 48 h. The duration of virus shedding was short, and by the third day after virus challenge

TABLE 1

Virus shedding in experimental A/Khabarovsk/77/H1N1 infection

Treatment group (*n*)	No. of subjects virus positive on treatment day:				
	1	2	3	4	Total
Aerosol rimantadine (12)	8	2	1	0	8
Oral rimantadine (12)	8	3	0	0	8
Placebo (12)	7	5	1	1	7

only one subject in the aerosol treatment group and one in the placebo group continued to shed virus. Seven volunteers given aerosol rimantadine, nine given oral rimantadine, and seven given placebo treatment showed fourfold or greater increases in serum HAI antibody to the challenge virus upon testing of paired sera.

Illness

Of the 12 placebo recipients, three had no apparent illness, nine developed respiratory symptoms, and four of these nine also had symptoms of constitutional illness. In the two drug groups, one half of the volunteers had no apparent illness, and one half developed respiratory symptoms. None of the aerosol group (P=0.05 vs. placebo, Fisher's exact test) and one of 12 receiving oral rimantadine (P=0.16) reported constitutional symptoms. As shown in Table 2, illness scores in the placebo group peaked on the second treatment day, approximately 48–72 h after virus challenge. This peak was not observed in either of the drug treatment groups. The placebo group had a mean total illness score that tended to be higher than that observed in either of the drug groups, but the range of illness scores was broad in all groups.

Similar results were observed when only the virus-positive volunteers were considered. On the second treatment day the mean illness score in placebo recipients was 5.3 (S.D.

TABLE 2

Illness scores in experimentally induced A/Khabarovsk/77/H1N1 virus infection

Treatment group (*n*)	Mean symptom score on treatment day					Mean ± S.D. total symptom score	(range)
	1	2	3	4	5		
Aerosol rimantadine (12)	0.0	0.5	0.6	0.8	0.3	2.1 ± 3.2	(0–11)
Oral rimantadine (12)	0.1	0.6	0.5	0.6	0.1	1.7 ± 2.0	(0– 5)
Placebo (12)	0.1	3.3[a]	1.0	0.7	0.2	5.3 ± 7.1[a]	(0–23)

[a] Placebo vs. aerosol or oral rimantadine; $0.05 < P < 0.1$, Mann–Whitney U test, one-tailed.

= 5.3) as compared to 0.6 (S.D. = 0.7) in aerosol rimantadine or 0.9 (S.D. = 1.2) in oral rimantadine recipients ($P<0.02$, placebo vs. either drug group, Mann–Whitney's U test, one-tailed). Similarly, the mean total illness scores were 7.6 (S.D. = 8.7), 2.6 (S.D. = 3.8) and 2.3 (S.D. = 2.2) in placebo, aerosol rimantadine and oral rimantadine-treated, virus-positive volunteers, respectively ($0.05 < P < 0.1$, placebo vs. either drug group).

Fever

Low grade febrile reactions (axillary temperature > 37°C) were more frequent in the placebo recipients (7/12) than in either aerosol (2/12, $P=0.05$ vs. placebo, Fisher's exact test) or oral (1/12, $P=0.03$) rimantadine recipients. On the second treatment day six out of 12 placebo subjects, compared to none of those in the drug groups have elevated temperatures (>37°C) ($P=0.01$ vs. either drug group, Fisher's exact test). On this day the mean maximal temperature for the placebo subjects was 37.0°C (range 36.7–38.4°C), as compared to 36.7°C (range 32.3–36.9°C) in each of the drug groups ($P<0.05$ placebo vs. either drug group, Student's t test). Overall, 9.4% of all temperature measurements were elevated in the placebo group, as compared to 1.9% in the aerosol-treated and 0.9% in the orally treated volunteers ($P<0.01$, placebo vs. either drug group, chi square test). Of virus-positive volunteers, two out of eight receiving aerosol rimantadine and none of them receiving oral rimantadine developed temperature elevation at some time after virus inoculation, as compared to six out of seven placebo recipients ($P=0.05$ vs. aerosol, $P<0.005$ vs. oral Fisher's exact test).

Toxicity

No clinically significant changes occurred in hematologic, biochemical, or spirometric values. The most common symptom associated with aerosol treatments was unpleasant taste or smell which was noted by seven volunteers treated with aerosol rimantadine, as compared to one placebo recipient ($P = 0.01$, Fisher's exact test) and none of those receiving oral rimantadine ($P<0.01$). Two placebo recipients and one volunteer in each drug group reported nasal irritation during aerosol treatments. These symptoms were mild and resolved within 10–15 min of the end of aerosol treatments. In no instance were they sufficiently prominent to interrupt or alter treatments.

DISCUSSION

We found that the therapeutic activity of aerosolized rimantadine was comparable to that of oral rimantadine in experimentally induced human influenza A virus infection due to an H1N1 subtype virus. Both treatments regimens tended to be more effective than placebo in attenuating symptoms of infection. Compared to placebo administration, both regimens were associated with significant reductions in the number and height of febrile reactions and with significant reduction in the peak illness scores of volunteers

with documented infection. Aerosol rimantadine treatment was also associated with a significant decrease in the number of volunteers reporting symptoms of constitutional illness.

No significant effects on infection rates were observed in the drug-treated groups, perhaps because drug treatment was not begun until 24 h after virus inoculation. Infections, as determined by virus isolation, occurred in only 58–67% of volunteers in each group, in part because volunteers with relatively high preinoculation serum HAI antibody titers were included. No significant effects on the duration of virus shedding were noted, possibly because of short duration of virus shedding observed in this study and because of the small numbers of infected volunteers. The trend for fewer number of virus isolations in the aerosol-treated subjects as compared to the placebo-treated could have been due to carry over of drug from the nasal secretions into the in vitro isolation system, since no special precautions were taken in this regard. Previous studies with other topically applied antivirals, such as enviroxime in rhinovirus infection [6] and ribavirin in influenza A virus infections [4] have demonstrated that residual drug in nasal secretions can interfere with virus isolation.

In our study we have used an attenuated, H1N1 subtype virus strain for infection, which probably contributed to the generally mild degree of illness and short duration of virus shedding. In contrast, in experimental influenza induced by intranasal installation of H3N2 subtype strains ($10^{3.0}$–$10^{5.0}$ $TCID_{50}$), earlier studies showed that an average of 59% (38–86%) of infected volunteers developed a febrile influenza syndrome and 14% (0–50%) developed non-febrile upper respiratory symptoms [1]. In naturally acquired A/USSR/77/H1N1 infection of college students, Van Voris and coworkers used a similar method of symptom analysis and observed mean peak symptom scores that were 6–8-fold higher than the peak scores observed in the placebo recipients in the current study [10].

Despite these limitations, the current study revealed that aerosol delivery of low doses of rimantadine was associated with a therapeutic effect that was comparable to that observed with larger dosages of oral rimantadine. No direct measurements of aerosol particle size or deposition were made, but the volunteers treated with aerosol rimantadine could have retained no more than the total administered dose (50 mg of rimantadine/day), in contrast with a total daily dosage of 150 mg in those receiving oral rimantadine. Ultrasonic nebulizers similar to the equipment used in that study usually produce aerosol particles with aerodynamic mass median diameter predominately in the 5–10 μm range. This means that in the current study high rates of drug deposition probably occurred in the upper respiratory tract and nasopharynx, although the actual retention rate was likely less than 100% of the administered dose [5].

Other recent clinical studies support our observation that direct delivery of anti-influenza drugs to the respiratory tract is at least as effective as oral administration. Although oral ribavirin at dosages of 1000 mg/day orally was not effective in treatment of naturally acquired influenza A/Brazil/78/H1N1 virus infection [7], Knight and co-

workers reported that ribavirin given in dosages of 400–600 mg/day by small particle aerosol shortened the duration of fever, symptoms, and virus shedding in influenza A/England/333/80/H1N1 infection [4]. The encouraging results observed in the current study provide impetus for further studies of aerosolized rimantadine treatment for naturally occurring influenza A virus infection.

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