ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1987, p. 1470–1473
0066-4804/87/101470-04$02.00/0

Vol. 31, No. 10

Protective Efficacy of Low-Dose Amantadine in Adults Challenged with Wild-Type Influenza A Virus

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Received 11 May 1987/Accepted 29 July 1987

The prophylactic efficacy of a low dose (100 mg) of amantadine hydrochloride against experimental challenge with influenza A/Texas/1/85 (H1N1) wild-type virus was determined in healthy adult volunteers in a placebo-controlled, double-blind, randomized trial. No side effects of the 100-mg dose were observed in the amantadine-treated volunteers. Compared with placebo, 100 mg of amantadine significantly reduced the frequency of illness (9 of 22 versus 2 of 22 volunteers, $P < 0.04$) and provided 78% protection against influenza illness. The two ill volunteers in the amantadine group had rhinitis only, whereas most of the ill placebo controls developed both systemic and upper-respiratory-tract illness. Wild-type virus was recovered from 50% of the amantadine-treated volunteers, compared with 82% of the placebo controls. Of note, the infected amantadine recipients shed 100 times less virus and shed virus for half as many days as did the infected placebo recipients. Although amantadine restricted viral replication, it did not interfere with the development of an antibody response to influenza virus. These results indicate that in adults experimentally challenged with influenza wild-type virus, 100 mg of amantadine is effective both in the prevention of influenza illness and in the restriction of virus replication.

Amantadine hydrochloride given as a 200-mg daily dose has been shown to be effective in preventing illness caused by influenza A virus (5, 10). Amantadine is recommended for short-term prophylaxis during influenza A outbreaks for high-risk patients who have not received influenza vaccine previously, during epidemics when influenza vaccine might be ineffective because of antigenic drift in the influenza virus, and to supplement protection of patients who may be expected to mount a poor antibody response to vaccination (2, 3). However, since central-nervous-system side effects have been associated with the 200-mg dose, amantadine has been underused for the prevention of influenza. Recently, the Immunization Practices Advisory Committee of the Centers for Disease Control and the Food and Drug Administration have advocated decreasing the dose of amantadine to 100 mg daily in persons 65 years of age or older because renal function normally declines with age and side effects may occur more frequently in the elderly with a daily dose of 200 mg (2).

Although a few studies (16–18) of naturally occurring influenza illness have suggested that the low dose of amantadine (100 mg) may be as effective as a 200-mg dose in preventing influenza illness in younger adults, only one study (19) of experimental challenge with influenza virus has demonstrated the efficacy of this lower dose. However, in this study volunteers were not selected according to preinoculation antibody status, and the overall rate of documented influenza infection was low. We therefore conducted a placebo-controlled, double-blind, randomized study to determine the efficacy of low-dose (100-mg) amantadine in adults challenged with wild-type influenza A virus. In addition, we evaluated the effect of low-dose amantadine on the magnitude and duration of influenza virus replication in the challenged volunteers.

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MATERIALS AND METHODS

Volunteers. Study protocols were approved by the Institutional Review Board of the Francis Scott Key Medical Center, the Joint Committee on Clinical Investigation of the Johns Hopkins Hospital, and the Medical Research Group of the Pharmaceutical Division of E. I. du Pont de Nemours & Co., Inc. Healthy adults, 18 to 40 years of age, who had a hemagglutination-inhibition antibody (HAI) titer in serum less than or equal to 1:8 for influenza A/Texas/1/85 (H1N1) virus were recruited from the Baltimore area. Volunteers were ineligible if they had a history of seizures, vaccination against influenza A (H1N1) virus, or allergy to amantadine, or if they were taking medications that might interfere with the study. Each volunteer gave written, informed consent.

Clinical studies. The volunteers participating in this trial were admitted to the Center for Immunization Research Isolation Unit at Francis Scott Key Medical Center during the summer and early fall of 1986. Volunteers were isolated for 3 days before and 10 days after virus inoculation. Each volunteer was randomly assigned to receive either 100 mg of amantadine or a placebo capsule identical in appearance once daily for 8 days; the study was conducted in a double-blind manner. The amantadine and placebo capsules were prepared, packaged, and coded by E. I. du Pont de Nemours & Co., Inc., of Wilmington, Del. On day 4 of medication, each volunteer was inoculated intranasally (0.25 ml per nostril) with a dose of $10^{6.7}$ 50% tissue culture infectious doses ($TCID_{50}$) of wild-type influenza A/Texas/1/85 (H1N1) virus. Volunteers were interviewed and examined daily by two physicians, and the oral temperatures and pulses of the volunteers were recorded four times a day. To assess drug toxicity, they were questioned daily about the development of central-nervous-system and gastrointestinal symptoms.

Volunteers were considered ill if they developed symptoms and physical findings consistent with influenza illness

TABLE 1. Protective effect of 100 mg of amantadine hydrochloride compared with placebo in 44 volunteers challenged intranasally with $10^{6.7}$ $TCID_{50}$ of influenza A/Texas/1/85 (H1N1) wild-type virus

Treatment (no. of subjects)	% Infected[a]	% with indicated illness			
		Febrile or systemic	Upper respiratory tract	Lower respiratory tract	Any
Amantadine (22)	77	0	9	0	9[b]
Placebo (22)	91	23	41	4	41

[a] Infection was defined as virus isolation, antibody response, or both.
[b] $P < 0.04$ (chi-square test).

within 9 days after virus inoculation. Illnesses were categorized by meeting one or more of the following criteria: fever (oral temperature of ≥37.8°C taken twice); systemic illness (occurrence of myalgia alone or with chills and sweats); upper-respiratory-tract illness (rhinitis, pharyngitis, or both for 2 or more consecutive days); and lower-respiratory-tract illness (persistent cough for 2 consecutive days). An illness was attributed to influenza when confirmed by virus isolation.

Virus. Influenza A/Texas/1/85 (H1N1) clone 1-1 (lot E-245) wild-type virus was administered to volunteers. It was grown in the allantoic cavity of specific-pathogen-free eggs (SPAFAS, Inc., Norwich, Conn.) by L. Potash (Flow Laboratories, Inc., McLean, Va.). The virus suspension was safety tested for the presence of adventitious agents by L. Potash; none were found. The same lot of virus (E-245) and dose of $10^{6.7}$ ($TCID_{50}$) were used in each of three challenge studies.

Laboratory studies. Nasal wash specimens for isolation of wild-type virus were collected before challenge and daily for 10 days afterwards. The virologic methods have been previously described (13). Serum specimens were collected before and 3 weeks after virus administration for measurement of serum antibodies by HAI and enzyme-linked immunosorbent immunoglobulin G (IgG) hemagglutinin (HA)-neuraminidase (NA)-specific assays (14, 15). Paired serum specimens were tested by HAI assay using two antigenically related wild-type viruses as antigens: influenza A/Texas/1/85 (H1N1) and A/Chile/1/83 (H1N1). The antigen used in the enzyme-linked immunosorbent assay was a fraction of viral protein containing the HA and NA of the influenza A/Texas/1/85 (H1N1) virus prepared as described elsewhere (9). The antigens for the HAI assay and ELISA were kindly provided by Mark H. Snyder and Brian R. Murphy (National Institute of Allergy and Infectious Diseases, Bethesda, Md.). Infection was confirmed by virus isolation, a significant (fourfold) rise in serum antibody titer, or both.

Amantadine hydrochloride peak and trough levels were determined by standard methods (7) on blood collected at four intervals: immediately before the first dose, 2 h after dose 1, 2 h after dose 7, and immediately before dose 8.

Statistical analysis. Student's t test, the chi-square test with Yates correction, and the Fisher exact test were done where appropriate. The reduction in rate of illness in vaccinees (the efficacy rate) was calculated as follows: (rate of illness in placebo control group − rate in amantadine group) × 100/rate of illness in placebo control group.

RESULTS

A total of 45 volunteers were enrolled in the study, but 1 volunteer in the amantadine group inadvertently received placebo on one day. This volunteer did not develop clinical illness but shed virus for 4 days. These data have been excluded from analysis. The rates of influenza illness were significantly less in amantadine recipients than in placebo recipients (2 of 22 versus 9 of 22, $P < 0.04$) (Table 1). As compared with placebo, 100 mg of amantadine reduced the rate of influenza illness by 78%. The nine ill placebo recipients developed typical influenza illness, whereas the two ill amantadine recipients developed only mild rhinitis, which persisted for 2 to 3 days.

There was a tendency toward a lower frequency of infection with wild-type virus in amantadine recipients than in placebo controls, after challenge, but the differences were not significant (Table 1). Among the 22 amantadine recipients, 14 (64%) had significant increases in ELISA IgG titers, and 16 (73%) had increases in HAI antibody titers in serum after challenge (Table 2). The frequency of serum HAI and ELISA IgG antibody responses in the placebo controls was 91%. Among those who had a serum antibody response, there was no difference in the magnitude of antibody responses between the amantadine group and the placebo controls.

The effect of amantadine on virus replication was assessed by comparing the magnitude and duration (i.e., number of days) of virus shedding during the first 5 days after virus challenge (when volunteers were taking the drug) and the subsequent 5 days after the drug was stopped (Table 3). Five of the amantadine recipients shed influenza virus after amantadine was discontinued, as did eleven of the placebo recipients. The magnitude of virus shedding in infected amantadine recipients was reduced significantly both during the treatment period ($P < 0.003$) and after the treatment period ($P < 0.03$) as compared with that in infected placebo controls. The number of days of virus shedding in infected amantadine recipients was also significantly reduced during the treatment period ($P < 0.005$) but not during the posttreatment period.

Mean blood levels of amantadine were 165 ng/ml (range, 95 to 321 ng/ml) 2 h after the initial dose, 444 ng/ml (range, 125 to 941 ng/ml) at steady-state peak, and 172 ng/ml (range,

TABLE 2. Immune response of seronegative volunteers given 100 mg of amantadine or placebo before and after challenge with $10^{6.7}$ $TCID_{50}$ of influenza A/Texas/1/85 (H1N1) wild-type virus[a]

Treatment (no. of volunteers)	Serum HAI			Serum ELISA IgG[b]		
	Titer		% with increase	Titer		% with increase
	Pre	Post		Pre	Post	
Amantadine (22)	2.7 ± 0.9	5.3 ± 1.6	73	9.7 ± 1.4	11.7 ± 1.4	64
Placebo (22)	2.8 ± 0.9	5.6 ± 1.4	91	9.0 ± 2.3	12.6 ± 1.7	91

[a] Antibody titers are expressed as reciprocal mean $\log_2$ titers ± standard deviations. Pre refers to preinoculation and post refers to postinoculation with wild-type virus.
[b] The antigen used in the ELISA was a fraction of protein containing the HA and NA of the influenza A/Texas/1/85 (H1N1) virus.

TABLE 3. Virological results in adult volunteers treated with 100 mg of amantadine hydrochloride or placebo 3 days before and 5 days after intranasal challenge with $10^{6.7}$ $TCID_{50}$ of influenza A/Texas/1/85 (H1N1) wild-type virus

Treatment (no. infected)	No. shedding virus	During 5-day treatment period		During 5-day period after treatment	
		Total days of virus isolation (mean ± SD)[a]	Mean peak titer (log_{10} $TCID_{50}$/ml ± SD)[a]	Total days of virus isolation (mean ± SD)[a]	Mean peak titer (log_{10} $TCID_{50}$/ml ± SD)[a]
Amantadine (17)	11	1.2 ± 1.4[b]	1.29 ± 0.97[c]	0.4 ± 0.7	0.74 ± 0.45[d]
Placebo (20)	18	3.5 ± 1.7[b]	3.01 ± 1.92[c]	0.7 ± 0.7	1.61 ± 1.42[d]

[a] Data from infected volunteers were used for calculations. The lowest detectable quantity of virus shed was 0.75 $TCID_{50}$/ml. Culture-negative samples were assigned a value of 0.50 $TCID_{50}$/ml for purposes of calculation.
[b] $P < 0.005$ (two-tailed Student's t test).
[c] $P < 0.003$ (two-tailed Student's t test).
[d] $P < 0.03$ (two-tailed Student's t test).

17 to 749 ng/ml) at steady-state trough. The steady-state peak (125 ng/ml) and trough (7 ng/ml) levels of one ill volunteer were very low, whereas the other volunteer who became ill had high levels (863 ng/ml peak and 749 ng/ml trough).

DISCUSSION

Influenza is a major cause of morbidity and mortality despite the availability of effective vaccines and the antiviral drug amantadine hydrochloride (4). Controlled trials have shown that a 200-mg daily dose of amantadine is effective for prophylaxis against and treatment of illness caused by influenza A virus, yet the drug is underused for influenza prevention (5, 10, 11, 19–21). This is caused, in part, by concern about drug toxicity; central-nervous-system symptoms occur in approximately 5 to 10% of recipients (4, 7, 8). Side effects with the 200-mg dose appear to occur more frequently (or are noticed more often) in the elderly, the population at highest risk for severe influenza illness (1–3). For this reason, a 100-mg dose has been recommended by the Centers for Disease Control and recently approved by the Food and Drug Administration for influenza prophylaxis in persons over 65 years of age (2).

Our placebo-controlled study demonstrated that a 100-mg dose of amantadine reduced clinical illness in young adults after experimental challenge with wild-type influenza A/Texas/1/85 (H1N1) virus. The protective efficacy of the 100-mg regimen was 78%. Although our study did not compare 100- and 200-mg doses, the level of efficacy (78%) that we found with the 100-mg regimen was comparable to the efficacy (70 to 90%) reported for 200 mg of amantadine in previous experimental- and natural-challenge studies in adults (5, 10, 11). One study by Younkin et al. (21) demonstrated that 100 mg of amantadine had therapeutic efficacy equal to that of 200 mg. Other studies (16–19) that have assessed the prophylactic effectiveness of 100 mg of amantadine have been difficult to interpret. Smorondintsev et al. (18) in the Soviet Union reported that, compared with placebo, 100 mg of amantadine significantly reduced serologically confirmed cases of influenza illness during an outbreak; however, they excluded 20% of the participants from the analysis. In another study (19), Smorondintsev et al. found 100 mg of amantadine to be effective in preventing influenza illness after experimental challenge, but volunteers were not randomized by preinoculation antibody status, viral isolation was not attempted, and there was a very low rate of serologically documented influenza infection. In two other studies (16, 17), 100 mg of amantadine given daily to boys in English boarding schools during an outbreak of influenza was effective in reducing the number of cases of influenza illness. Neither study, however, included a placebo group, and, in one, most of the subjects had been vaccinated previously.

In closed studies with experimental challenge, quantitation of the duration and magnitude of virus shedding is a useful assessment of the ability of an antiviral agent to restrict virus replication in vivo. There is a strong positive correlation between the level of influenza virus replication and development of illness which follows challenge with wild-type virus (4, 12). Clearly, an anti-influenza drug that restricts virus replication should lessen the likelihood of transmission as well as prevent illness. To assess the effect of amantadine on virus replication, we collected and cultured nasal washes daily from all volunteers after virus inoculation and measured the quantity of virus recovered. Our results indicate that, compared with placebo, amantadine reduced the number of days of virus shedding and reduced the quantity of virus shed by half. We observed no rebound in virus shedding and no increase in clinical illness after amantadine was discontinued. In contrast, another study showed a rebound in virus shedding after rimantadine, an analog of amantadine, was discontinued (6). The reasons for this difference are not clear.

Recent recommendations for the control of influenza from the Immunization Practices Advisory Committee of the Centers for Disease Control state that the daily dose of amantadine for persons 65 years old or older should be reduced from 200 mg to 100 mg daily to minimize toxicity (2). In an uncontrolled study conducted in a nursing home during a recent outbreak of influenza A (H3N2) disease (1), 100 mg of amantadine was used to prevent the spread of influenza virus and was associated with a low incidence of side effects, suggesting that the guidelines of the Centers for Disease Control may be appropriate. The 100-mg dose regimen of amantadine did not cause side effects in the young adults in our study. The absence of side effects may be due to the lower levels in blood achieved with the 100-mg dose as compared with the levels for the 200-mg dose, reported to be 40% higher (7, 8). The lower levels in blood with the 100-mg dose may account for the reduced toxicity in the elderly. Since 100 mg of amantadine was effective in preventing influenza illness after experimental challenge, field trials with 100 mg of amantadine may be warranted to determine whether this regimen is effective against naturally occurring influenza A illness.

ACKNOWLEDGMENTS

This research was supported by a grant from E. I. du Pont de Nemours & Co., Inc.

We thank Judy Abelow, Sharon Kanellopulos, Paul Kilmon, Bruce Thomson, and the nursing staff from the Center for Immuni-

zation Research for invaluable help in the recruitment of volunteers and for special assistance in the conduct of the study; Howard Hoffman of the Drug Metabolism Group, Du Pont Co., who kindly performed the amantadine assays; Karen Christina and Paulo Miotti for laboratory assistance; and Dyer Bilgrave and Carla Talley for secretarial support.

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