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Amantadine Therapy of Epidemic Influenza A_2 (Hong Kong)[1]

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In a double-blind comparison of the therapeutic effect of amantadine in a natural outbreak of Hong Kong influenza in January 1969 near Houston, Texas, the decrease in titers of virus in throat swabs from 12 treated patients during the first 10 hr of treatment was appreciably greater than similar titers of virus from 16 untreated patients ($P = 0.06$). The titer of shed virus decreased significantly more rapidly ($P < 0.05$) among amantadine-treated patients ill more than 48 hr before treatment than among five control patients who had been ill more than 48 hr. Cough, sore throat, and nasal obstruction cleared more rapidly in treated patients ($P < 0.05$), and decline of fever in 6 treated patients sick for less than 48 hr before treatment was more rapid than among 13 untreated patients who had been ill for less than 48 hr. These findings are considered to be consistent with a limited therapeutic effect of the drug.

An opportunity to test the therapeutic efficacy of amantadine hydrochloride in Hong Kong influenza was afforded when an outbreak of this disease occurred in January 1969 among inmates of two neighboring institutions of the Texas Department of Corrections. In a double-blind comparison of clinical, virological, and serological responses, amantadine-treated patients exhibited a more rapid reduction in the titer of virus recovered from throat swabs, a more rapid clearing of some clinical manifestations, and, in those patients ill less than 48 hr before start of treatment, defervescence was more rapid. The present report describes details of this study.

MATERIALS AND METHODS

Patients. Participants were adult male inmates of the Ramsey I and II units of the Texas Department of Corrections, who, when seen at sick call, volunteered to join the study. They were transferred to either of two hospital wards which were situated at Ramsey II.

Design of study. Before treatment, a complete history was taken of each patient, and he was given a complete physical examination, urinalysis, complete blood count, and throat culture for bacteria. An X-ray of the chest, made on one subject with rales, was negative. Throat cultures for pathogenic bacteria were repeated once during treatment. Blood counts were repeated twice. All patients received one dextropropoxyphene (Darvon) capsule (65 mg) on the night of admission; eight patients received dextropropoxyphene the next evening, but thereafter placebo was given when analgesics were requested.

The study was arbitrarily limited to 29 of 37 ill volunteers who shed the Hong Kong strain of virus and who showed a fourfold or greater neutralizing antibody response to the agent. Eight were not included; four of the patients were ill with neither virus-shedding nor antibody response. Two showed serological responses but without virus shedding, and from two of the patients there were single virus isolations but no antibody response. A single positive culture for a rhinovirus was obtained for one patient (Brown). From one patient not included in the study, we obtained a single positive culture for a rhinovirus 1 day after he showed a positive culture for the Hong Kong variant of influenza virus. One patient, who showed a rise in antibody titer but no positive cultures for influenza virus, shed herpes simplex virus in a single culture. Patients ranged in age from 22 to 42 years. The average ages of the treated and of the control groups were 27 and 29 years, respectively. None of the subjects had received influenza vaccine for at least 1 year and infrequently, if at all, before that. Illness had persisted before treatment for an average period of 42 hr in amantadine-treated patients and 36 hr in placebo-treated patients. The differences were not significant (t test).

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Medication. Amantadine hydrochloride in 100-mg capsules or placebos of an identical appearance were given at 12-hr intervals for at least 6 days. [Amantadine hydrochloride (Symmetrel) and placebos were provided by E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.] Patients were classified by degree of fever at the time of admission: up to 101, 101.1 to 102, 102.1 to 103, and 103.1 F or higher. Drug or placebo was assigned within these groups by a random method. Sixteen patients received placebo and 13 received drug.

Oral temperatures were recorded at 2-hr intervals from 8:00 AM to 4:00 PM, at 8:00, 10:00, and 12:00 PM, and at 4:00 AM, a total of nine readings each study day.

Physicians examined the patients twice daily and recorded (according to a format prepared by E. I. du Pont de Nemours & Co., Inc.) an evaluation of the severity of the following symptoms and signs, on the basis of a 1 to 3+ scale. *Symptoms:* feverishness, headache, chills or chilliness, sweats, malaise and fatigue, myalgia, joint pain, eye pain, nasal stuffiness or discharge, sore or scratchy throat, laryngitis, dry cough, productive cough, chest wall pain, substernal pain, and shortness of breath. *Signs:* apathetic appearance, conjunctivitis, hyperemic nasal mucosa, nasal mucosal swelling, pharyngitis, tonsillitis, altered fremitus, rales or rhonchi, and muscular soreness.

Virus isolation and identification. Throat swabs were agitated in tubes containing 3 ml of veal infusion broth with 0.5% bovine albumin and antibiotics (i.e., penicillin and streptomycin), pressed free of excess liquid, and discarded. The tubes were stored in wet ice and, within 3 hr, 0.4 ml of the fluid from each was inoculated into a rhesus monkey kidney culture. Specimens were incubated at 36 C and tested for virus by the hemadsorption procedure with 0.1% guinea pig erythrocytes (2). At least 32 to 100 50% tissue culture infective doses $(TCID)_{50}$ of the first and last isolates from each patient were tested against 20 antibody units of chicken antiserum prepared against each of the following strains of influenza virus: A_2/Aichi/2/68, A_2/Taiwan/1/64, A_2/Japan/170/62, and B/Singapore/3/64. Neutralization occurred only with the antiserum prepared against the Aichi strain.

The quantity of influenza virus in throat swabs was determined by inoculation of 0.2-ml amounts of 10-fold serial dilutions of original specimen into each of two tubes of rhesus monkey kidney culture. Hemadsorption was tested for at 5 and 10 days, and virus titers were calculated by the Spearman-Kaerber method.

Neutralizing antibody. The titer of neutralizing antibody was measured in rhesus monkey kidney cultures, with influenza A_2/Hong Kong/68 (406th U.S. Army Laboratory no. 8138558) used as the test strain, by a method described elsewhere (1).

RESULTS

Clinical. Recovery from illness was rapid in both groups of patients and corresponded closely with defervescence. There were no complications, but a few patients noted persisting or recurring moderate cough and malaise. These symptoms gradually disappeared.

TABLE 1. *Degree of significance of lesser severity of signs and symptoms in treated versus control patients*[a]

Signs and symptoms	*U*	*P*
Nasal stuffiness	32	<0.01
Productive cough	51	<0.02
Dry cough	54	<0.05
Sore, dry throat	59.5	0.05
Joint pain	60	0.06

[a] Wilcoxon test.

Within each treatment group, severity of symptoms and, to a lesser extent, severity of signs, tended to be concordant in individual volunteers. Thus, if one symptom was mild in degree, other symptoms were mild. The degree of concordance was highly significant. [*Friedman test for concordance.* Symptoms: volunteers receiving placebo, $\chi^2 = 34.9$, 15 degrees of freedom (DF), $P < 0.01$; amantadine treatment, $\chi^2 = 47.7$, 12 DF, $P < 0.01$. Signs: control group, $\chi^2 = 27.6$, 15 DF, $P < 0.05$. Treatment group, $\chi^2 = 21.1$, 12 DF, $P < 0.05$.]

Generally, signs and symptoms were less severe in amantadine-treated patients. Therefore, despite the lack of independence of their degree of severity, it was of interest to make comparisons between treated and control groups. From among the symptoms and signs used to evaluate the severity of illness, four (Table 1) were significantly less severe in treated patients, and a fifth was of borderline significance.

In a further analysis, we calculated the number and percentage of patients in each group who exhibited each of the symptoms and signs. By using as a basis the 18 signs or symptoms exhibited by at least 60% of patients in each group, we determined the time required, in successive 12-hr periods, for a 50% reduction in severity. This was done for the entire groups, for those ill more than 48 hr, and for those ill less than 48 hr before treatment. In no instance were differences between amantadine-treated and placebo-treated groups statistically significant, although there was a uniform trend toward less severity in amantadine-treated groups.

Fever. The excess of temperatures over 98.8 F, recorded nine times daily for 7 days, was calculated and summed. The totals of summed excesses in each group were compared and found not to be significantly different ($U = 65$, $P > 0.10$).

Temperature responses in the two groups were also compared with time required to return to 99

and 100 F. For example, all amantadine-treated patients returned to persisting levels of < 99 F by 84 hr, at which time only 53% of patients receiving placebo had reached this level. A similar trend was observed in time required to return to ≤ 100 F. Nevertheless, neither difference was statistically significant. The average time required by the amantadine-treated patients to reach persisting levels of 99 F or less was 44.5 hr, whereas the time required by the control group was 71.3 hr. This difference was statistically significant (t test, $P < 0.05$). The only other point of significant difference with respect to defervescence was a more rapid return to ≤99 F in the 6 treated patients who had been ill for less than 48 hr at the time of treatment in comparison to 13 other patients who had been ill for less than 48 hr but were given placebo (100% versus 38% at 72 hr, χ^2 value = 4.21, $P = < 0.05$).

TABLE 2. *Virological and serological finding according to treatment with amantadine or placebo*

Patient	Virus titer[a]					Neutralizing antibody titer (reciprocal)[b] at 14 days
	0 hr	10 hr	34 hr	58 hr	82 hr	
Amantadine-treated						
Bates	4.0[c]	3.0	2.0	0.5	1.0	16
Hol	4.5	4.0	0.5	1.5	0	128
Aug	5.5	0	0.5	0.5	0	512
May	2.5	3.0	3.0	0.5	0	16
Barr	2.5	0	0.5	0	0	256
Brown	3.0	0	0	0	0	256
Band	0	0.5	1.0	0.5	0	8
John	4.5	1.5	0	0	0	256
Slone	3.5	2.0	0.5	0	0	16
Stall	6.0	3.0	0	0.5	0	16
Thom. H.	0.5	0	2.0	0.5	0	16
How	4.5	1.0	0	0.5	0	4
Hunt	4.0	NS[d]	0	0	0	16
Placebo-treated						
Alex	4.5[c]	4.0	1.0	1.5	0	128
Umst	4.5	0.5	0.5	0	0	256
Jen	3.0	2.0	1.0	0	0	256
May	1.5	3.0	1.0	1.5	1.0	16
Pax	1.5	0.5	0	0.5	1.0	256
MacD	3.0	1.5	0.5	0	0	<2
Kel	1.0	0	2.0	0	0	256
Thom T.	4.5	3.5	0	0	0	16
You	3.0	1.0	1.0	0	0	64
Rober	4.5	4.0	1.0	0	0	64
Alv	2.5	2.5	4.5	3.0	NS	64
Bak	4.5	3.5	1.5	0.5	0	16
Wms	0	3.0	0.5	0	0	<2
Woods	1.0	0	0	0	0	16
Fraz	4.0	3.5	0.5	1.0	0	64
Rich	4.5	4.0	0	0.5	0	256

[a] Log_{10} per 0.1 ml of swab fluid. The geometric mean titers in amantidine-treated patients were: 3.46 at 0 hr, 1.50 at 10 hr, 0.77 at 34 hr, 0.39 at 58 hr, and 0.076 at 82 hr. The geometric mean titers in control patients were: 2.97 at 0 hr, 2.28 at 10 hr, 0.94 at 34 hr, 0.53 at 58 hr, and 0.13 at 82 hr. Percentages of positive specimens in amantadine-treated patients were: 92% at 0 hr, 67% at 10 hr, 62% at 34 hr, 62% at 58 hr, and 8% at 82 hr. Percentages of positive specimens in control patients were: 94% at 0 hr, 88% at 10 hr, 75% at 34 hr, 44% at 58 hr, and 13% at 82 hr.

[b] Titer before treatment was 2, except for Barr and Jen (no specimen) and Rober (1:16).

[c] Fifty per cent tissue culture infectious doses.

[d] NS, not significant.

Virus shedding. Throat swabs obtained before

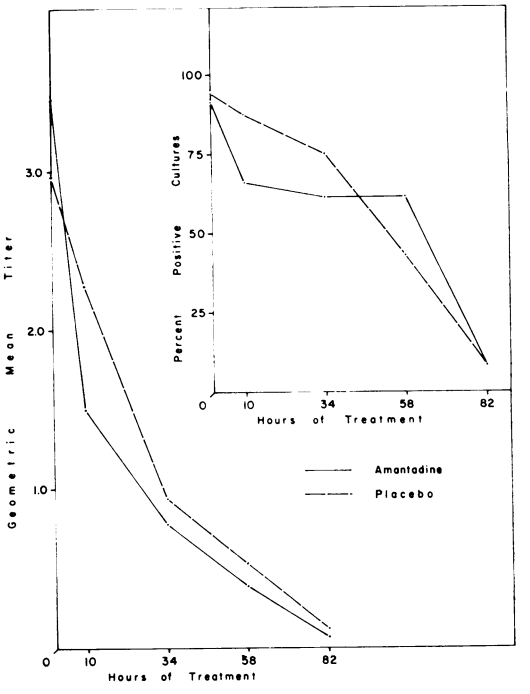

FIG. 1. *Changes in titer of virus and frequency of positive cultures from throat swabs in patients according to treatment with amantadine or placebo.*

TABLE 3. *Mean log titer change at 0 to 10 hr in patients receiving amantadine or placebo*

Group	No. of volunteers	Mean log titer change[a]	Std error	Range
Control	16	−0.69	0.37	−4.0–3.0
Amantadine	12[b]	−1.92	0.52	−5.5–0.5

[a] $P = 0.06$, $t = 1.98$.

[b] One man excluded because of no specimen at 10 hr.

treatment, at 10 hr after start of treatment, and at three subsequent 24-hr intervals were titered for influenza virus. The average titers and the frequency of positive cultures, according to treatment or placebo administration, are shown in Table 2 and Fig. 1. Despite a general trend of fewer positive cultures and lower titers of virus shed by treated patients, the differences approach significance only in the reduction in titer in the interval from before treatment to 10 hr after start of treatment (Table 3, $P = 0.06$). When comparisons were made among patients in treated groups and control groups, each divided into those who were ill for more and those who were ill for less than 48 hr before medication, it was found that the five amantadine-treated patients who were ill for more than 48 hr showed a significantly greater reduction in virus titer in the first 10 hr than did the other five patients who were ill for more than 48 hr and who were given placebo [$t(8) = 2.31, P < 0.05$]. Differences were not significant among patients ill less than 48 hr.

Antibody response. The geometric mean rise in neutralizing antibody from onset of illness to 14 days was 32-fold in 13 amantadine-treated patients and 64-fold among 16 volunteers who received placebo; this difference was not significant ($t = 1.0, P < 0.20$).

Tolerance to therapy. There were no gastrointestinal or other reactions suggesting intolerance to the drug. Some blood counts and urine examinations showed abnormalities consistent with acute influenza, but returned to normal with recovery from illness. The bacterial flora of the nose and throat showed no shifts toward predominance of any particular species of microorganism, and there were no apparent differences in bacterial flora with reference to the use of amantadine.

DISCUSSION

In the present controlled study of amantadine therapy of naturally occurring infection with the Hong Kong variant of influenza virus, quantitative clinical, virological, and serological studies revealed differences in favor of a therapeutic effect of amantadine which approached or achieved significance. In treated patients ill for more than 48 hr before treatment, there was a more rapid decline in the amount of virus shed, some respiratory signs and symptoms cleared more rapidly, and there was a less rapid decline in fever than in patients ill less than 48 hr. These findings are consistent with the concept that amantadine produces a limited therapeutic effect. The differences in response of patients who were ill more than 48 hr and those who were ill less than 48 hr probably do not have special significance, except that they indicate the borderline effectiveness of the drug.

The action of the drug is presumably primarily antiviral, since five isolates from this epidemic were susceptible to <1 μg of drug per ml of medium in vitro (C. E. Hoffman, E. I. du Pont de Nemours & Co., Inc., *personal communication*), and it was active against influenza A_2/AA/2/60, A_2/Bethesda/10/63 (3), and A_2/Hong Kong/50/68 (C. E. Hoffman, *unpublished data*) infections in mice when treatment was begun 48 to 64 hr after initiation of infection. Moreover, experience in other outbreaks of human A_2 influenza showed more rapid recovery in patients given amantadine (1*a*, 5).

The present dose of amantadine was selected because it is the recommended dose of the drug for prophylactic use. Since reported side effects have to do primarily with the level of consciousness and physical performance, it was necessary that prophylactic use did not create a hazard of accidents. Patients ill with influenza should invariably be at rest, however, and some degree of drug intolerance would be acceptable if, through an increased dose, an improved therapeutic effect would result. In view of the apparently complete absence of intolerance to the present dose, therapeutic studies with larger doses should be undertaken. Rimantadine, a derivative of amantadine, has also shown promising activity in laboratory [oral antiviral agent, EXP 126 (rimantadine hydrochloride), Laboratory Studies, October 1966, Investigational Brochure, E. I. du Pont de Nemours and Co.] and clinical studies (4), and further studies with this agent would be of interest.

The widespread loss of time from work and school makes epidemic influenza a serious occupational hazard; however, the greatest concern is the mortality which accompanies outbreaks. The chief cause of mortality is influenzal pneumonia with and without its bacterial complications, and, although the pathogenesis of this syndrome is not well understood, it should be a target of antiviral therapy. No opportunity was presented to study such cases in the present outbreak, but it is conceivable that a drug of even limited therapeutic activity, when given early in illness, might prevent the pulmonary dissemination of the virus with its ominous prognosis. Since it is difficult to identify early those patients in whom complicating pneumonia will occur, a difficult tactical problem in clinical medicine is created by the availability of a drug of limited effectiveness. Further study will be needed to resolve this problem.

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