THERAPEUTIC EFFICACY OF AMANTADINE HCl AND RIMANTADINE HCl IN NATURALLY OCCURRING INFLUENZA A2 RESPIRATORY ILLNESS IN MAN*

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Abstract In a double-blind, placebo-controlled study of the therapeutic efficacy of amantadine HCl (1-adamantanamine HCl) and of rimantadine HCl (α-methyl-1-adamantanemethylamine HCl) in patients with naturally occurring respiratory disease due to influenza A2, carried out at the Virginia State Penitentiary during an influenza A2 outbreak in January, 1968, both drugs were found to be effective therapeutically. Statistically significant increases in the rate of overall clinical improvement, in the rate of defervescence and in the rate of disappearance of signs and symptoms of illness were observed in the patients who received amantadine HCl or rimantadine HCl as compared to those who received placebo.

THE prophylactic anti-influenza activity of amantadine HCl† has been demonstrated in the laboratory with the use of tissue-culture, egg and mouse model systems infected with a variety of strains of influenza A, A1 and A2,[1-4] in horses "challenged" with influenza A/equine 2/Lexington/3/63,[5] in human volunteers with artificially induced influenza A2 infection[6-8] and in human volunteers exposed to natural infections with influenza A2.[9-12] Evidence for the therapeutic action of amantadine HCl has been reported in laboratory model systems,[2,13] including mice infected with influenza A2/Hong Kong/50/68,[14] and possibly in patients exposed to a natural infection of influenza A2.[10] An opportunity to study further the therapeutic effect for amantadine HCl in man occurred during an outbreak of influenza A2 respiratory illness at the Virginia State Penitentiary in January, 1968. This is a report of that study.

Included in the study was an analogue of amantadine HCl, α-methyl-1-adamantanemethylamine HCl (rimantadine HCl), which had been shown to have prophylactic and therapeutic activities in laboratory-model infections of influenza A2 similar to those of amantadine HCl.[15] Prophylactic activity was shown in human volunteers "challenged" with influenza A2/Rockville/1/65.[16] The analogue was included in the present study to demonstrate any therapeutic activity it might have against influenza A2 in man.

MATERIALS AND METHODS

Conduct of Study

During the influenza A2 outbreak, inmates with influenzal symptoms were admitted to the study if the illness had developed suddenly within the previous 24 hours. Participation was voluntary after the purpose of the study had been explained. On admission, a complete medical work-up, including the ratings of the severity of 17 signs and 22 symptoms on a scale of 0 to 4 (absent, mild, moderate, severe and very severe) was done. During the next five days the examination was repeated twice daily at 8 a.m. and 4 p.m. Oral temperature, pulse and respiration readings continued to be taken every four hours except at 1 a.m. By the fifth day of the study most patients had recovered sufficiently to be released on a "cell pass"; however, they continued to report to the hospital twice daily for five days more to be examined. The few patients who had not recovered sufficiently by the fifth day were kept in the hospital as long as necessary. Complete hospital care was provided as needed.

Amantadine HCl (100 mg), rimantadine HCl (150 mg) and lactose as placebo were provided in identical hard gelatin capsules in randomly distributed individual coded vials in sufficient amount for one patient for 10 days. The medication was administered personally under double-blind conditions by the hospital nurse starting at the time of admission and was continued on a twice daily basis at 8 a.m. and 4 p.m. for 10 days. Concurrent medication was prescribed as needed, but antipyretics were not administered.

Laboratory Methods

Laboratory specimens were collected routinely for bacteriological examination on the first day, for virus isolation on the first and second, for white-cell and differential counts on the first through the fifth, for diagnostic serology on the first, tenth and twenty-first and for urinalysis on the first and tenth days. X-ray films of the chest were taken on the first and fifth days.

Virus isolations from the pharyngeal swab specimens were carried out by amniotic infection of 10-day-old embryonated eggs with the use of chicken erythrocytes as the indicator of 48-hour hemagglutinin formation. A maximum of five serial passages were made before absence of virus was established. Identification of the isolates was made by hemagglutination inhibition with the use of influenza A2/Japan/305/57 and influenza A2/Georgia/1/67 specific antiserums. Sensitivity of the homologous virus influenza A2/Virginia/1/68 to amantadine HCl

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†In the form of Symmetrel, E. I. du Pont de Nemours & Co., Wilmington, Del.

and rimantadine HCl was determined by following hemagglutinin production in the allantoic cavity of drug-treated and control nine-day embryonated eggs. Neutralizing antibody titers were determined on heat-inactivated acute-phase and convalescent-phase serum samples with the use of 30 to 100 $TCID_{50}$ of influenza A2/Virginia/1/68 in a hemadsorption assay on rhesus-monkey-kidney cells.

Evaluation of Data

The therapeutic effectiveness of amantadine HCl and rimantadine HCl was evaluated by means of three indexes: the rate of overall clinical improvement; the rate of defervescence; and the rate of disappearance of signs and symptoms of illness. All the patients with confirmed influenza A2 respiratory illness were used to evaluate the data on signs and symptoms. However, not all patients entered the study with a temperature elevated to 100°F or greater. Only those whose oral temperatures were 100°F or greater on the first day of the study were used to evaluate the temperature data; they were also used to evaluate the overall clinical improvement because of its dependence on both temperature and signs and symptoms of illness. Statistical analyses included chi-square analysis with correction for continuity, rank analysis using the Mann-Whitney U test,[17] probit analysis[18] and principal-component analysis.[19]

Results

Demographic Data

From a total population of approximately 1400 inmates, 95 patients who met the necessary criteria were admitted to the study from January 3 to February 8, 1968.

After the drug code was broken at the completion of the study, it was found that 48 patients had received placebo, 23 had received amantadine HCl and 24 had received rimantadine HCl. Confirmation of influenza A2 was made in all but one of the 95 patients with the use of serologic conversion (fourfold or greater rise in neutralizing antibodies at 21 days) and virus isolation as evidence. The comparability of the three groups of patients is indicated in Table 1. Though such factors were not included, the three groups were also comparable in racial distribution, the number entering the study in the morning and in the afternoon, the time of day they received their first medication and their influenza vaccination record.

The illnesses were typical of those due to influenza A2 viruses[20] and were considered to be moderate to severe in degree. There was broad constitutional involvement (apathetic appearance, malaise and fatigue, chills, myalgia, headache, muscular soreness, feverishness, sweats and joint pain). Locally involved were the eyes (complaints of burning sensation and light sensitivity, with evidence of conjunctivitis and watery appearance), nose (congestion and sneezing, together with hyperemia and swelling of the mucosa and discharge) and throat (complaints of soreness with evident pharyngitis). The chest was not involved to any great extent except for some pain and a dry cough. Anorexia and abdominal tenderness were seen frequently, but nausea and vomiting were rare.

Overall Clinical Improvement

The effect of amantadine HCl and rimantadine HCl on the rate of overall clinical improvement was evaluated on the basis of the 79 patients with confirmed influenza A2 who had oral temperatures of at least 100°F on the day they entered the study (39 placebo patients, 20 receiving amantadine and 20 receiving rimantadine HCl). These patients were classified as having shown rapid, medium or slow clinical improvement. For rapid improvement, the temperature had to show a reduction to 100°F or less within 24 hours of the start of medication, together with 50 per cent or more clearing of all the signs and symptoms within the first 36 hours. If either of these criteria were not met, the patient was considered as having improved at a medium or slow rate according to the duration of the 100°F temperature (24 to 36 hours, or longer than 36 hours, respectively). A comparison of the distributions of the patients given placebo, amantadine HCl and rimantadine HCl according to these rates of clinical improvement (Fig. 1) reveals that there

TABLE 1. *Group Comparability.*

MEDICATION	NO. OF SUBJECTS*	CRITERION†					
		AGE (YR)	WEIGHT (LB)	ACUTE SERUM NEUTRALIZING ANTIBODY TITER‡	INTERVAL FROM ONSET OF ILLNESS TO START OF MEDICATION (HR)	TEMPERATURE AT START OF MEDICATION (°F)	COMPOSITE SIGNS & SYMPTOMS AT START OF MEDICATION§
Placebo	47	33.8 ± 8.6	173.0 ± 27.5	3.5 ± 1.9	15.3 ± 7.9	101.0 ± 1.6	68.4 ± 19.5
Amantadine HCl	23	34.4 ± 11.4	168.2 ± 18.8	3.5 ± 1.7	15.7 ± 6.3	101.4 ± 1.5	76.5 ± 17.2
Rimantadine HCl	24	32.0 ± 9.3	164.9 ± 27.8	3.5 ± 2.3	15.6 ± 6.8	100.8 ± 1.5	67.0 ± 16.5

*With confirmed influenza A2.

†Mean ± SD.

‡Reciprocal of geometric mean, $\log_2$.

§Mean total score of severity ratings of all signs & symptoms.

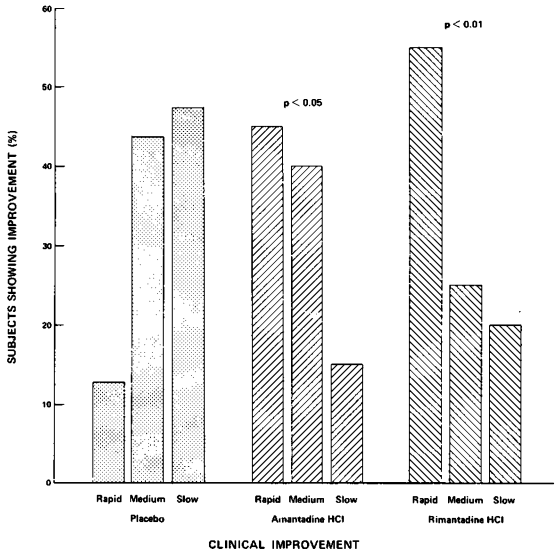


FIGURE 1. *Effect of Amantadine HCl and Rimantadine HCl on the Percentage of Patients Showing Rapid, Medium and Slow Rates of Clinical Improvement (Probability by Chi-Square Analysis).*

was a statistically significant increase in the number of rapidly improving patients in each of the drug-treated groups as compared to the placebo group (p less than 0.05 and p less than 0.01 by chi-square analysis).

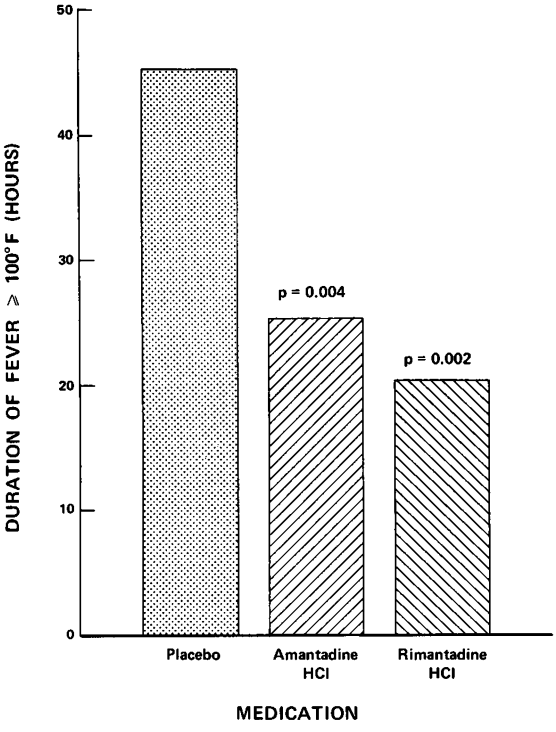
Fever

The effect of amantadine HCl and rimantadine HCl on the duration of fever was examined by comparison of the time of the last recording of a temperature of at least 100°F that was associated with the acute influenzal illness (Fig. 2). Treatment with both amantadine HCl and rimantadine HCl shortened by statistically significant amounts the duration of temperatures in excess of 100°F (22 and 28 hours respectively).

The consistency of this response is demonstrable in the number of patients whose temperatures fell to less than 100°F at progressive intervals after medication was started (Fig. 2). Significant numbers of patients given amantadine HCl and rimantadine HCl showed a more rapid defervescence sooner than the placebo patients did. By probit analysis the median time for the temperatures of the amantadine HCl patients to fall to less than 100°F was 23 hours as compared to 45 hours for the placebo group (p less than 0.01). Nineteen hours were required by those receiving rimantadine HCl (p less than 0.01).

Signs and Symptoms

The effects of amantadine HCl and rimantadine HCl on the rate of disappearance of signs and symptoms of illness were evaluated with the use of the intervals from the start of medication to the point of a 50 per cent reduction (a perceptible determination) in the composite illness-severity scores (Fig. 3). Treatment with amantadine HCl and rimantadine HCl significantly shortened the time required for this degree of improvement (15 and 18 hours re-



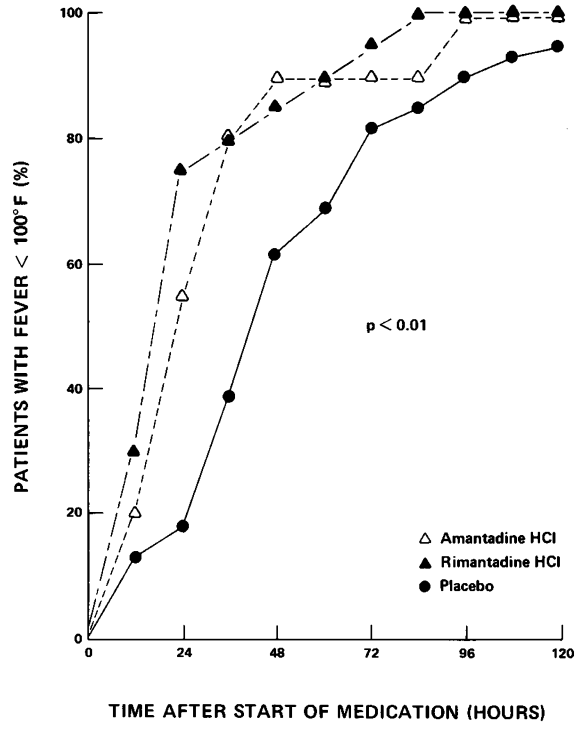


FIGURE 2. *Effect of Amantadine HCl and Rimantadine HCl on the Duration of Fever (Temperature over 100°F) and on the Percentage of Patients with Fever (Temperature under 100°F) after the Start of Medication (Probability by Rank and Probit Analyses Respectively).*

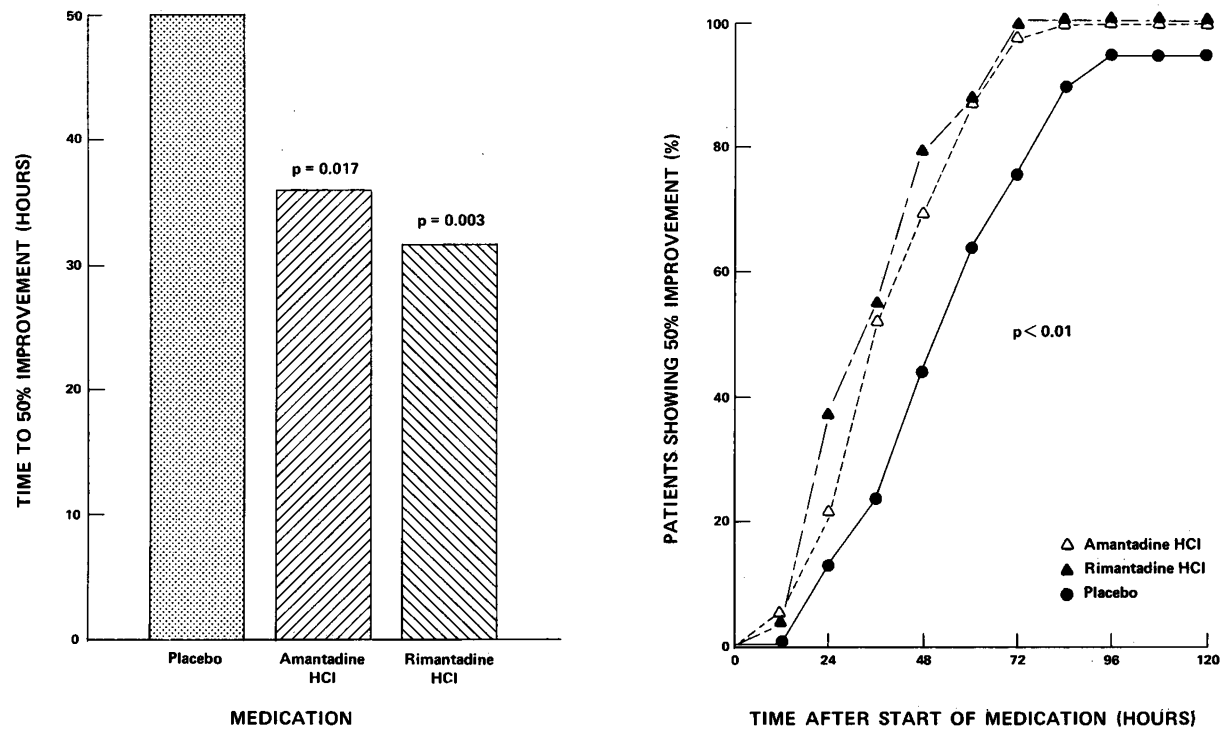


FIGURE 3. *Effect of Amantadine HCl and Rimantadine HCl on the Time Required for a 50 per Cent Improvement in Composite Signs and Symptoms of Illness, and on the Percentage of Patients Showing 50 per Cent Improvement after the Start of Medication (Probability by Rank and Probit Analyses Respectively).*

spectively). Of the patients who achieved 50 per cent recovery at progressive intervals after medication was started (Fig. 3), significantly more of those treated with drug recovered sooner than the placebo patients. By probit analysis the median time for the amantadine HCl patients to show such improvement was 35 hours, as compared to 49 hours for the patients given placebo (p less than 0.01). Thirty-one hours was required by the patients treated with rimantadine HCl (p less than 0.01).

The effects of the two drugs were evaluated further during the acute phase of the illness on the basis of the percentage of patients free of 11 selected signs and symptoms consistent with influenzal illness during the first four days of the study (Fig. 4). The signs and symptoms included dry cough, swelling of the nasal mucosa, sore throat, nasal congestion, eye pain, myalgia, malaise and fatigue, headache, apathetic appearance, chills and feverishness. Although significantly more of the amantadine HCl patients had all these signs and symptoms at the start of medication, treatment with the drug resulted in a significant increase over the placebo group of patients who were free of such signs and symptoms starting late in the afternoon of the second day. The patients given rimantadine HCl were comparable to those receiving placebo at the beginning of the study. They also responded to treatment, so that significantly more were free of these signs and symptoms starting early the morning of the second day.

The above responses of the composite signs and symptoms to medications are supported by the statistically significant observation that each of the

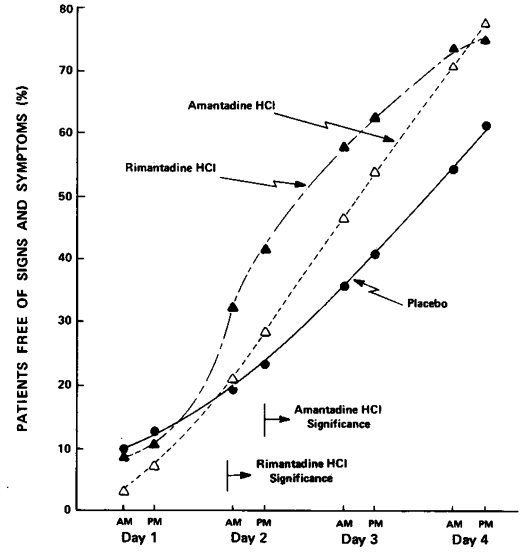


FIGURE 4. *Effect of Amantadine HCl and Rimantadine HCl on the Percentage of Patients Free of 11 Signs and Symptoms (Listed in the Text) Consistent with Influenzal Illness during the First Four Days of Medication (Significance by Principal-Component Analysis).*

more frequent signs and symptoms disappeared sooner in the drug-treated than in the placebo-treated patients (binomial sign test).

Laboratory Results

Influenza A2 virus was isolated from one or both of the pharyngeal specimens of 93 of the 95 patients who entered the study. The two patients who failed to shed virus were in the placebo group. There were no differences between the virus-shedding patterns of the patients given placebo and those of the drug-treated patients during the two-day observation period. Confirmation of the identity of the first virus isolate was made by Dr. Marion Coleman, of the National Communicable Disease Center, with the official designation of influenza A2/Virginia/1/68. The sensitivity of this virus to both drugs was established by the absence of hemagglutinin in the allantoic cavity of 48-hour infected embryonated eggs, which had been pretreated 30 minutes before infection with 500 μg per egg of amantadine HCl or rimantadine HCl. By comparison, the allantoic fluid of infected control eggs had a geometric hemagglutinin titer of 1:12.

Serologic conversion was demonstrated in 35 of 48 patients given placebo (73 per cent), 19 of 23 given amantadine HCl (83 per cent), and 21 of 24 given rimantadine HCl (88 per cent) (differences not significant by chi-square analysis). The degree of 21-day antibody rise was similarly unaffected by either of the two drugs. Whereas the geometric mean neutralizing-antibody rise of the patients given placebo was 27.8, those of the patients receiving amantadine HCl and rimantadine HCl were 22.7 and 36.8, respectively (differences not significant by rank analysis).

There were no significant blood, urine or x-ray changes during the study. Bacteria regarded as etiologically important were not isolated from any of the patients.

Side Effects

No complaint attributable to amantadine HCl or rimantadine HCl was observed during the 10 days of dosing.

Discussion

The relatively short duration of a self-limiting disease, such as uncomplicated respiratory illness due to influenza A2, and the subjective nature of the signs and symptoms used to follow the course of the disease demand carefully controlled clinical studies for the proper evaluation of the effectiveness of a therapeutic regimen. The patients used in the evaluation must have confirmed influenza, and the groups must be comparable in presenting illness and the factors that might affect the course of the disease. The groups must be large enough to minimize the effect of inherent variation, and the evaluation of the resulting data must encompass the multifacets of an infectious respiratory illness. Each of these requirements has been handled in the present study with the result that both amantadine HCl and rimantadine HCl have been shown to be effective therapeutically in patients ill with uncomplicated upper-respiratory-tract disease due to influenza A2.

That both drugs, in contrast to the placebo, resulted in more rapid rates of overall clinical improvement, defervescence and disappearance of the signs and symptoms of illness reinforces the conclusion that a beneficial effect was observed with each of the drugs alone. When consideration is given to the chemical similarity of amantadine HCl and rimantadine HCl and to the similarities of their antiviral activities, including the recent reports of therapeutic activity in patients with influenza A2,[10,21,22] the effects observed in the present study are not unexpected.

The present data do not permit conclusions about the relative potencies of the two drugs. The patients who received rimantadine HCl appeared to improve somewhat more rapidly than those who received amantadine HCl. The fact that the two drugs were used at different dosages, 100 mg per capsule for amantadine HCl and 150 mg per capsule for rimantadine HCl, and that the signs and symptoms of illness of the patients receiving amantadine HCl were more prevalent than those of the patients treated with rimantadine HCl may account for this apparent difference.

Just what the mode of action is whereby these drugs exerted their therapeutic effect can only be inferred from the knowledge at hand. Symptomatic relief of the illness does not appear to be responsible for the beneficial effects observed. Amantadine HCl has been shown to be free of the analgesic and antihistamine-like properties and probably free of the antipyretic properties necessary for such relief.[23] Rimantadine HCl has been shown to be free of all these properties.[24] In man amantadine HCl and rimantadine HCl have had no effect on the course of disease of viruses known to be resistant to the antiviral action of the drug.[25,26] On the other hand, laboratory evidence indicates that both drugs possess antiviral activity against influenza A2 that is exerted through an inhibition of virus penetration into the susceptible cell.[27] Although this suggests that their action is prophylactic and not therapeutic, at least for the individual cell, therapeutic action would still be possible for a group of cells if the drugs were administered early enough after a low multiplicity of infection to protect the cells not yet infected. This hypothesis finds application in the therapeutic demonstration of the drugs in the experimental animal-model infections and in patients ill with upper-respiratory-tract disease due to influenza A2 as observed in the present study. It also suggests that virus multiplication is continuing during

the period of acute illness, a conclusion that is supported by the shedding of virus observed during the first two days of the study. The failure of either drug to prevent this shedding may be related to the qualitative nature of this procedure as well as the brief interval that was studied.

The lack of effect of the two drugs on antibody formation appears to be in contrast to the reduced antibody formation that follows prophylactic treatment[8,16] but is in agreement with the failure of amantadine HCl to influence the formation of antibodies in mice after bacteriophage injection.[28] The observations are compatible, however, when the timing of medication in relation to the antigenic mass needed for stimulation of the antibody-synthesizing system is taken into account.

That amantadine HCl and rimantadine HCl were able to improve significantly the rate of recovery of previously healthy adult males with uncomplicated upper-respiratory-tract disease due to influenza A2 is of practical value. By their antiviral chemotherapeutic effect during the acute phase of the illness, the period of convalescence and absence from normal activities should be influenced favorably, as should the frequency of secondary complications, which tend to be so serious in susceptible persons. Furthermore, the demonstration of therapeutic activity, together with the previously established prophylactic activity, indicates that beneficial effects may be expected from these medications irrespective of the time of exposure to infection.

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