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Activity of Amantadine, Rimantadine, and Ribavirin Against Swine Influenza in Mice and Squirrel Monkeys

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Amantadine, rimantadine, and ribavirin given orally, either prophylactically or therapeutically, reduced mortality and increased the survival time of 3-week-old mice infected with the type A/New Jersey/8/76 (swine) strain of influenza virus. In addition, amantadine and rimantadine, administered therapeutically, increased the rate of virus clearance from lungs of infected mice. Administration of amantadine either before or after virus challenge ameliorated the illness in squirrel monkeys; when administered therapeutically, it appeared to eliminate virus shedding from infected monkeys within hours after therapy was initiated.

The outbreak of influenza in early 1976, caused by a virus serologically related to the "swine" virus implicated in the 1918-19 pandemic, stimulated an accelerated national vaccination program (10). As part of this program, our laboratory evaluated several candidate vaccines on the basis of protective efficacy in laboratory animal models and evaluated chemoprophylactic and chemotherapeutic drugs in animal models. The results of studies to evaluate the vaccines will be reported separately.

Amantadine, rimantadine, and ribavirin have demonstrated activity against disease caused by several strains of type A influenza virus (6, 8, 9, 12, 13). This paper reports results of studies on the efficacy of these three drugs given by the oral route for the prevention and/or treatment of type A/New Jersey (swine) influenza virus infections in mice. Amantadine, which is the only one of these drugs currently approved for use in humans, was also evaluated in a squirrel monkey model for influenza virus infection (4).

MATERIALS AND METHODS

Mice. Outbred, female Swiss mice (Crl:COBS CD1[ICR]BR) were obtained from the "Sendai-free" colony of Charles River Laboratories, Wilmington, Mass. Two ages of mice were used in separate experiments, weanlings (21 days old) and adults (6 to 8 weeks old). Mice were randomly selected, and a maximum of 20 to a cage were housed in biological containment cabinets operated under negative pressure. Lighting in the cabinets was controlled to give 12 h of light and 12 h of darkness each day. Commercial mouse pellets and water were provided ad libitum.

Monkeys. Male squirrel monkeys (*Saimiri sciurius*), weighing 0.5 to 0.9 kg, were used. Housing and feeding arrangements have been described (4).

Virus. Influenza virus, strain A/NJ/8/76 (Hsw1 N1), with a history of six passages in embryonated eggs, was adapted to mice in nine serial passages. Mice were infected by intranasal instillation of supernatant fluid obtained by centrifugation of a suspension of homogenized lungs removed from mice infected 3 to 4 days previously. After passage 9, allantoic cavities of 10-day-old embryonated chicken eggs were inoculated with supernatant fluid from infected lungs. After incubation for 48 h at 35°C, infected allantoic fluid was harvested and clarified by centrifugation at 1,200 × *g* for 15 min at 4°C. Antibiotics were added to the clarified fluid to achieve a final concentration of 250 U of penicillin per ml and 100 μg of streptomycin per ml; aliquots of the suspension were stored at −60°C. Titrations in embryonated chicken eggs indicated that the infected allantoic fluid contained $10^{7.7}$ egg median infectious doses (EID_{50}) of virus per ml.

Drugs used and treatment schedule. Amantadine hydrochloride and its structural analog, rimantadine hydrochloride, were obtained from E. I. duPont de Nemours and Co., Inc., Newark, N.J. Ribavirin was obtained from the Nucleic Acid Research Institute of ICN Pharmaceuticals, Inc., Irvine, Cal. The single-dose oral 50% lethal dose of these drugs for mice has been estimated to be 700 mg of amantadine per kg (14), 739 mg of rimantadine per kg (C. E. Hoffman, personal communication), and 6,400 mg of ribavirin per kg (R. W. Sidwell, personal communication). For the present study, the drugs were dissolved at a concentration of 0.25 mg/ml in sterile distilled water and given to the appropriate groups of mice in lieu of drinking water as previously described by McGahen et al. (9). New stock solutions were prepared weekly, and fresh drug solution was added to the drinking vessels daily. Treatment was initiated at selected times beginning as early as 48 h before or as late as 96 h after challenge with infectious virus. Treatment was discontinued 14 days postinfection in all studies. Based on preliminary experiments, daily consumption of drug solution was estimated at 6 ml per mouse. On this basis, each treated mouse ingested approximately 60 mg of drug per kg of body weight per day, although this value may have varied for acutely ill mice.

Amantadine was administered to monkeys by

means of a nasogastric tube connected to a syringe and passed through a steel speculum inserted between the monkey's teeth and into the esophagus. Two doses of amantadine were tested, 7.5 mg/kg per day and 15.0 mg/kg per day. A 0.5-ml volume containing one-half of the prescribed dose was given at 8 a.m. and the balance at 1 p.m. each day. Infected control monkeys were given the same volume of sterile water. Treatment, which was continued for a total of 7 days in each study, was initiated either 24 h before virus challenge or 48 h after challenge, a time when clinical illness was apparent.

Virus challenge. Mice, lightly anesthetized with ether, were given 10^6 EID_{50} of virus in 0.05 ml by the intranasal route. Monkeys were challenged with 10^7 EID_{50} of virus by the intratracheal route as previously described (2, 4).

Sampling and assay procedure. At selected intervals after infection, lungs removed from mice were scored for gross lesions, weighed, and assayed for virus by established procedures (11). Virus was isolated from monkeys by swabbing the oropharynx. The swabs were washed in 1.0 ml of heart infusion broth containing 50 μg of gentamicin per ml, 100 U of penicillin per ml, and 100 μg of streptomycin per ml; these samples were assayed for virus by established procedures (11).

Clinical determinations and illness scoring. Beginning at least 2 days before infection, the rectal temperature, hematocrit, total and differential leukocyte counts, respiratory rate, pharyngeal virus isolation, and body weight were determined once daily for each monkey beginning at 8 a.m. Food consumption, nasal discharge, coughing and sneezing, labored breathing, and activity for monkeys were recorded at approximately 8-h intervals. To facilitate analysis of treatment effects, the system devised by Berendt and Hall (3) was employed to score the response of monkeys over the first 7 days of infection. With this system, a critically ill monkey would score approximately 77 (assuming a 20% weight loss and maximum values for the other parameters); sham-inoculated control monkeys scored <5.0.

RESULTS

Experiments in mice. Preliminary experiments revealed marked differences in survival between untreated weanling and adult mice following infection with the New Jersey strain of influenza virus. Intranasal doses of $10^{3.8}$ EID_{50} routinely killed one-half of the 21-day-old mice with a mean time to death of <6 days. In contrast, the 50% lethal dose for 6- to 8-week-old mice could not be determined. Highest lethalities (10%) were observed at a challenge dose of $10^{5.8}$ EID_{50}; survival rates were somewhat improved at higher doses, suggesting some type of interference phenomenon. Virus titers in the lungs of both weanling and adult mice exceeded 10^7 EID_{50} at 3 days; thereafter, lung virus concentrations gradually declined to undetectable levels by 9 to 11 days postinoculation. Extensive pulmonary consolidation and a significant increase in lung weight was observed by 6 days postchallenge.

Lung virus titers, lung lesion scores, and lung weights of adult mice infected with type A/NJ/influenza virus and given each of the drugs are summarized in Table 1. Virus replicated rapidly in the lungs of untreated mice, and the lungs of these mice weighed almost three times as much as those from noninfected mice. Approximately 40% of each infected lung had plum-colored lesions typical of influenza by day 7. Despite extensive pathological changes, however, 90 to 100% of the infected adult mice survived.

None of the drugs altered tissue virus levels measured 3 days postinfection. By 7 days, however, virus titers were significantly lower in mice treated therapeutically with ribavirin. The development of lung pathology, as reflected by lung weight, was less extensive when rimantadine was given prophylactically and when ribavirin was given therapeutically. Prophylactic administration of amantadine and rimantadine resulted in fewer lung lesions than were observed in untreated mice, but variation among lesion scores was too great to permit statistical discrimination.

In contrast to adult mice, type A/NJ influenza infections in untreated weanling mice were uniformly lethal in this study, with a mean time to death of 5.8 days (Table 2). Amantadine, rimantadine, and ribavirin used prophylactically de-

TABLE 1. *Effect of drugs given orally to 8-week-old mice infected with type A/NJ influenza virus*

Group	Lung virus titer[a] (log_{10} EID_{50}/infected lung)		Mean lung lesion scores[b] (7 days)	Mean lung wt (mg; 7 days)
	3 days	7 days		
Noninfected controls			0	142[c]
Prophylactic[d]				
Amantadine	7.7	5.8	0.9	310
Rimantadine	7.3	5.2	0.6	272[c]
Ribavirin	7.0	4.8	2.0	332
Therapeutic[e]				
Amantadine	6.8	4.2	1.2	324
Rimantadine	6.8	5.0	1.7	352
Ribavirin	7.1	3.6[c]	1.0	266[c]
Infected controls	7.1	5.5	1.7	390

[a] Geometric mean, five mice.
[b] Scale of 0 to 4 from negative to total consolidation.
[c] $P < 0.05$, compared with infected controls.
[d] Treatment initiated 24 h before virus challenge.
[e] Treatment initiated 16 h after virus challenge.

layed the time to death and significantly increased survival to 80 to 90%. None of the drugs studied significantly affected virus titers at 3 days in the lungs of infected mice. However, compared with untreated mice, treatment with either amantadine or rimantadine had significantly reduced lung virus titers by 7 days after virus challenge, suggesting that both drugs increased the rate of virus clearance from the respiratory tract.

In a separate study, the survival of infected weanling mice as a function of time when drug treatment was initiated relative to virus challenge was examined. These survival data (Fig. 1) clearly indicate that whereas early treatment was desirable, and survival rates declined as treatment was delayed, each of the drugs effectively reduced mortality rates even when treatment was delayed for as long as 4 days.

Experiments in monkeys. Preliminary experiments suggested that the activity of rimantadine on swine influenza differed little from that of amantadine. For this reason, and because amantadine is approved for use in humans by the Food and Drug Administration, we concentrated on the latter drug for primate studies.

Following intratracheal instillation of 10^7 EID_{50} of virus, monkeys became febrile within 24 h; fever then slowly subsided. Most other changes in clinical parameters reached a maximum in 2 to 5 days and then slowly returned to prechallenge values. Although there was considerable variation in the duration of convalescense, all clinical values approached normal by day 10. Illness scores for these infected, untreated monkeys averaged 45.9 in contrast to scores of <5 for uninfected monkeys.

Illness scores for infected monkeys treated with either 7.5 or 15.0 mg of amantadine per kg per day, beginning either 24 h before or 48 h after virus challenge, are shown in Table 3. The scores of treated monkeys were significantly lower than those of untreated monkeys, indicating that amantadine was effective both prophylactically and therapeutically. No clear-cut effect of dose was observed.

In an effort to determine the predominating drug effect, we subtracted the contribution made by virus shedding from the illness scores. Virus shedding was considered to be indicative of infection; the other parameters were signs of illness. After this adjustment, the average scores for all groups of treated monkeys were still lower than those calculated for controls, indicating that a major effect of the drug was a reduction in the severity of illness. Data on the effect of drug treatment on the duration of virus shedding are also summarized in Table 3. Prophylactically administered drug did not significantly alter the

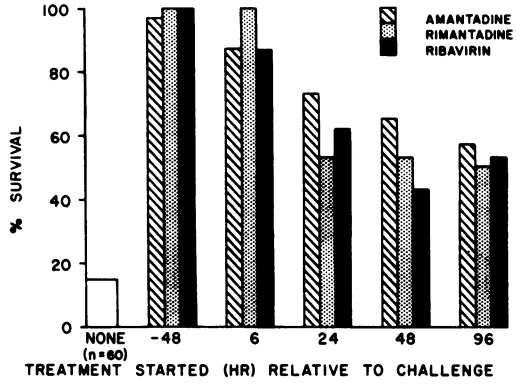

FIG. 1. *Effects of initiation time on drug efficacy for the treatment of type A/NJ influenza infection in mice.*

TABLE 2. *Effect of drugs given orally to 3-week-old mice infected with type A/NJ influenza virus*

Group	Lung virus titer[a] (log_{10} EID_{50}/lung)		Mean lung lesion score (7 days)	% Survival (n = 30)	Mean day of death
	3 days	7 days			
Prophylactic[b]					
Amantadine	7.7	6.0	1.4	83[c]	10.6[c]
Rimantadine	7.5	5.8	1.0	93[c]	10.5[c]
Ribavirin	7.4	6.0	2.6	90[c]	9.0[c]
Therapeutic[d]					
Amantadine	7.2	4.1[c]	1.0	63[c]	7.4
Rimantadine	7.3	4.2[c]	1.5	83[c]	6.0
Ribavirin	6.6	5.2	2.8	43[c]	5.3
Infected controls	7.4	5.8	3.0	0	5.8

[a] Geometric mean, five mice.
[b] Treatment initiated 24 h before virus challenge.
[c] $P < 0.05$ compared to controls.
[d] Treatment initiated 16 h after virus challenge.

TABLE 3. *Effect of orally administered amantadine upon illness scores and duration of virus shedding of squirrel monkeys infected with type A/NJ influenza virus*

Treatment	(n)	Dose (mg/kg) per day	Illness score	Adjusted illness score[a]	Days of virus shedding
Water control	8	0	45.0	38.9	5.75
Prophylactic	4	7.5	21.2[b]	15.4[b]	6.25
Prophylactic	4	15.0	23.4[b]	16.4[b]	4.50
Therapeutic (beginning at 48 h)	4	7.5	24.9[b]	20.9[b]	2.75[c]
Therapeutic (beginning at 48 h)	4	15.0	18.2[b]	14.7[b]	2.00[b]

[a] Total illness score less virus shedding contribution.
[b] $P < 0.005$ compared with water control.
[c] $P < 0.025$ compared with water control.

duration of virus shedding. Surprisingly, however, when treatment was initiated 48 h after virus challenge, the period of virus shedding was shortened significantly. Analysis of the other parameters that constitute the illness score revealed a lessening in all after prophylaxis or therapy rather than an effect on any particular one.

DISCUSSION

Amantadine, rimantadine, and ribavirin given orally, either prophylactically or therapeutically, reduced mortality and increased the mean time to death of mice infected with type A/NJ (swine) influenza virus. None of these drugs prevented infection, but amantadine and rimantadine therapy increased the rate of virus clearance from the lungs of young infected mice. In the present study, virus clearance in young mice was not significantly affected by oral ribavirin treatment. In contrast, previous reports (15) have attributed considerable antiviral activity to ribavirin administered as small-aerosol particles directly to the respiratory tract of infected animals. The reason for this discrepancy is not known, but may be due to the difference in drug level in the lungs after treatment by two different routes.

As previously reported (3), the New Jersey strain of influenza virus caused a milder illness in mice and squirrel monkeys than that observed in our laboratory after infection with an H3N2 serotype virus (11); the observation of mild illness in experimental animals is consistent with the report of Beare and Craig following the intranasal inoculation of humans (1). Amantadine treatment initiated either before or after virus challenge ameliorated the illness, and therapeutically administered amantadine apparently stopped virus shedding from infected monkeys within hours after treatment was started. Successful amantadine therapy for A/NJ virus infections in both mice and monkeys contrasts with the reports of other workers that therapeutically administered amantadine has only a minimal effect on the shedding of other strains of influenza virus (5). This observation suggests that the NJ strain may be even more susceptible to amantadine than other type A viruses, especially during stages of the infection when the virus is replicating rapidly. The increased survival seen in groups of mice in which treatment was not initiated until 96 h, and the dramatic cessation of virus shedding from infected monkeys when treatment was initiated at 48 h after virus challenge, suggest that amantadine need not be limited to a prophylactic role in influenza. The fact that amantadine-treated monkeys did not shed virus deserves special attention. Any reduction in virus dissemination from infected individuals could, of course, curtail epidemic spread of the virus.

Clearly, the therapeutic efficacy of amantadine cannot be explained wholly on the basis of antiviral activity. In our animal models, peak virus titers in the lung were often achieved before treatment was started. It is possible that the host's response to the drug played an important role in ameliorating the illness. This is consistent with findings by Little et al. (7), who observed that amantadine treatment increased the rate of recovery from disease in small airways and improved lung function in individuals suffering from naturally acquired influenza infections. Although none of the drugs prevented infection, amantadine reduced the severity of illness in monkeys, and all three drugs significantly increased survival of mice even when treatment was initiated after the onset of bronchopneumonia. The beneficial effect of treatment, obtained in two widely differing animal models, gives strong support to the hypothesis that these drugs might also be effective in treating influenza infections in humans.

Although vaccination continues to be the most widely used prophylaxis against influenza, immunological prevention and control of the

disease is not wholly adequate. Because of the capacity of influenza virus to undergo mutations that circumvent specific immunity established through vaccination with previously prevalent strains, vaccines are usually only partially protective. A need for effective therapeutic measures remains. This study supports the mounting evidence that amantadine, rimantadine, and ribavirin used alone or in conjunction with vaccine prophylaxis might offer better management of influenza than can be expected through vaccination procedures alone.

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