

Rimantadine Therapy of Influenza A Infection in Mice

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Eighty per cent of mice infected with a mouse-adapted strain of influenza virus died within 3 to 8 days after infection. Rimantadine given at maximum protective doses reduced mortality to 10%. This protection is dose related and can be demonstrated with doses from 4.5 to 24 mg per kg per day. Significant survival rates are shown by delaying treatment as long as 48 hr after infection. Lungs of treated mice have significantly less virus than those of controls at 24, 48, and 72 hr after infection. Antibody production as measured by hemagglutination inhibition is not different in treated and controlled mice. These results indicate that rimantadine is an effective prophylactic and therapeutic agent and that its activity is associated with decreased viral titers.

Amantadine and rimantadine have prophylactic activity against infections by many strains of influenza A virus (1, 5). Comparative studies in mice suggest that rimantadine was more effective than amantadine (11). Human experiments during spontaneous epidemics (10) or with induced disease (2, 6, 12) have also demonstrated that rimantadine decreases the severity and duration of the disease. Dose-response studies with amantadine by McGahen (7) demonstrated that mice had an increase in survival rate and survival time when compared with controls.

The present studies were done in an attempt to quantitate survival rate and infectious particles in mice given increasing doses of rimantadine. Rimantadine was chosen because of its known effect (11) in decreasing pulmonary virus titers when compared with amantadine.

MATERIALS AND METHODS

Mice. Sutter Farms male white Swiss mice weighing about 26 g were used in all experiments except in those designed to measure antibody production when the initial weight averaged 33 g. After light ether anesthesia, the mice were inoculated intranasally with 30 times the 50% lethal dose (30 LD₅₀). Lungs were removed aseptically at various time intervals and homogenized as a 10% suspension in phosphate buffer solution with antibiotics. Titers of infectious virus were measured by inoculation of 10-fold dilutions of ground lung suspensions into 10-day-old chick embryos. Titers are expressed as the 50% egg infective dose (EID₅₀).

Virus. Influenza A2/Jap/305, obtained from J. L. Schulman, Mt. Sinai Hospital, New York, was passed

28 times in mice and employed as the challenge virus in all experiments.

Treatment. Rimantadine dissolved in water was given subcutaneously twice daily in 0.2-ml volumes. Controls were injected with water in a similar manner.

Hemagglutination inhibition (HI). Blood from the mice was obtained by cardiac puncture (3), and their serological responses to influenza were measured by HI with 4 HA units of antigen by the microtiter method. The HI antibody titers were determined in individual mice. The results are reported as mean of groups of 10 mice with standard deviations.

RESULTS

Eighty per cent of control mice died between 3 and 8 days after infection. Thirty-six hours after infection the mice appeared ill. The fur was ruffled and the mice were huddled in a corner. Water and food consumption decreased (Fig. 1 and 2) and weight reduction was easily quantitated (Fig. 3). Infected nontreated mice lost weight progressively until death. Infected mice treated with 24 mg of rimantadine per kg per day had an initial decrease in weight followed by a weight increase at a rate parallel to that of the controls. By the end of the experiment, they had not reached the same weight as the noninfected animals. Mice given rimantadine and not infected continued to gain weight, drink, and eat as the normals.

Mice treated with rimantadine had increased survival rates (Fig. 4), which were dose related between 4.5 and 24 mg of rimantadine per kg per day. Further increase in survival rate with

36 mg of rimantadine per kg was not achieved. At the higher dose employed, 90% of the mice survived as compared with 20% of the controls. With doses of 4.5 mg per kg per day, 40% of the animals survived. When the treatment was begun with 24 mg of rimantadine per kg per day 1 hr after infection, the survival rate was 90%; the survival rate was 60% and 50%, respectively, when treatment was delayed for 24 and 48 hr after infection (Fig. 5). When treatment was delayed for 72 hr, there were no differences between treated or the control mice.

Lung virus content. The infectious virus content of the lungs is shown in Table 1. Untreated mice had 10^4 EID₅₀ of virus per g of tissue at 20

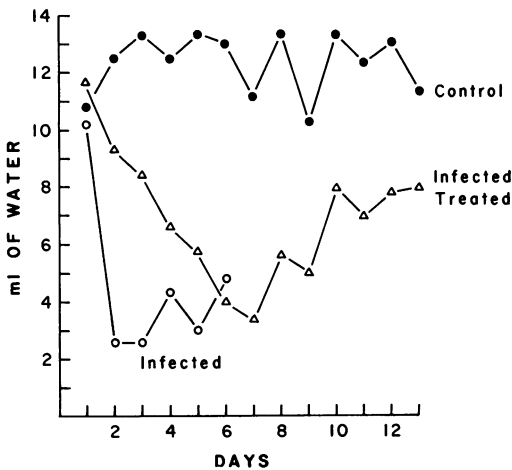


FIG. 1. Daily consumption of water in groups of 10 mice after influenza infection compared with uninfected controls and mice infected and treated with 24 mg of rimantadine per kg per day.

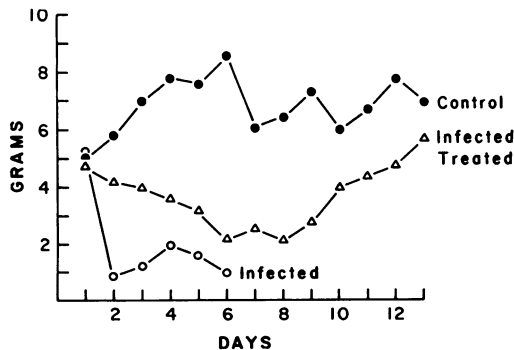


FIG. 2. Daily consumption of food in groups of 10 mice after influenza infection compared with uninfected controls and mice infected and treated with 24 mg of rimantadine per kg per day.

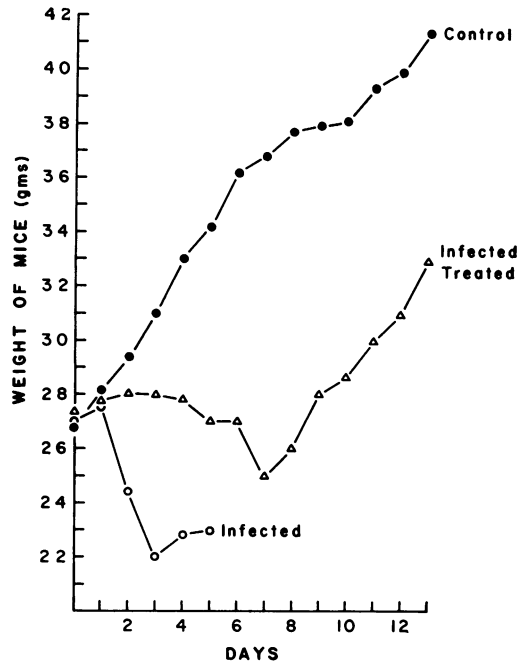


FIG. 3. Average weight of groups of 10 mice after influenza infection compared with uninfected controls and mice infected and treated with 24 mg of rimantadine per kg per day.

min after infection. Thereafter, these control mice had $10^{7.4}$, $10^{8.5}$, and $10^{8.5}$ EID₅₀ per g of tissue at 24, 48, and 72 hr, respectively, after infection. Reduction of two to three logs of virus was achieved with rimantadine doses of 9, 18, and 36 mg per kg per day, with the maximum reduction occurring at 48 hr.

Antibody production. The mean and standard deviation of HI antibody present are shown in Table 2. No significant differences were found between the control and treated groups.

DISCUSSION

Evaluation of antiinfluenza agents needs to be tested eventually in man. This evaluation is difficult because it is partially based on subjective symptomatology, subjective evaluation of physical findings, and antibody production. There are now several (2, 6, 10, 12) reports concerning the effect of rimantadine and amantadine in the prophylaxis and therapy of induced or spontaneous influenza in man. More objective data can be obtained from animals. McGahan showed that the first clinical evidence of infection in mice is decreased consumption of water (8). In the present study, we have shown that there is also a decreased consumption of food. As a

consequence mice lose weight. Measuring the weight of mice appears to be a simple and objective means to follow early infection.

In our model of mouse infection, 30 LD₅₀ produces an 80% mortality in 7 to 9 days. Rimanta-

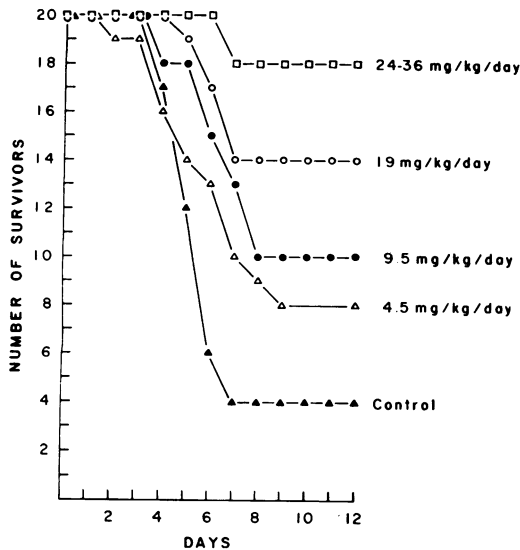


FIG. 4. Effect of increasing doses of subcutaneous rimantadine given in two daily doses to survivors after intranasal influenza A infection. Survivors with 24 and 36 mg per kg per day were identical. Treatment was started 2 hr after infection.

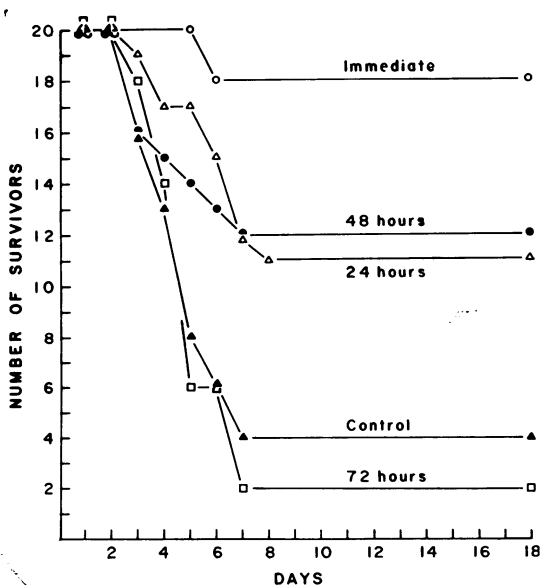


FIG. 5. Effect of delay in initiation of treatment with rimantadine on survivors after intranasal influenza A₂ infection. All mice received 24 mg of rimantadine per kg per day in two divided doses.

TABLE 1. Titration of eight pooled mouse lungs^a

Rimantadine (mg per kg per day)	Time after infection (hr)			
	24	48	72	96
36	-5.5	-5.5	6.0	6.5
18	-6.0	-6.5	-6.0	6.3
9	-7.3	-6.6	-6.7	6.7
0 (20 min, -4.0)	-7.4	-8.5	-8.5	Too few mice alive

^a Pulmonary virus titers were measured in eight mice from each group. Results are stated as the 50% egg infective dose, log 10; inoculum, 10^{6.0}.

TABLE 2. Effect of varying doses of rimantadine on the hemagglutination inhibition (HI) titer in the serum of mice 2 weeks after infection and treatment

Rimantadine (mg per kg per day)	Treatment at time of infection	
	HI (log 2 ± SD)	
36	7.6 ± 0.9	
18	7.7 ± 2.8	
9	6.6 ± 1.6	
4.5	6.5 ± 1.5	
0	7.0 ± 0.0	

dine at the maximum protective dose of 24 mg per kg per day decreased the mortality to 10%. Below this dose of rimantadine, mortality was shown to be dose related if given soon after infection. In addition, rimantadine therapy could be delayed for 48 hr after initiation of infection, with significantly decreased mortality. In addition, we have shown that the decreased mortality is associated with decreased viral titers in the lungs of mice. We cannot eliminate the possibility that other factors such as interferon or antibodies might have helped in the recovery of mice. Antibodies and amantadine have been shown to have added protection against influenza. In the experiment reported here, rimantadine did not interfere with antibody production as measured by HI. Therapy was started after the infection. Francis (4) showed, 30 years ago, that nasal secretions have a neutralizing activity on influenza virus. In human trials when amantadine and rimantadine are used as prophylactic agents, antibody production is decreased but not suppressed [6, 10, 12]. When rimantadine has been used in humans 24 hr after the initiation of symptoms, the amount of serum antibody has not differed from the controls. The influenza strain employed in the experiment reported here is

highly sensitive to amantadine (9). The results obtained with this sensitive strain cannot be generalized to other influenza A strains. Models such as used in this study are therefore needed to correlate with human therapeutic trials.

ACKNOWLEDGMENT

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LITERATURE CITED

1. Davies, W. L., R. R. Grunnert, R. R. Haff, J. W. McGahen, E. M. Neumayer, M. Paulshock, J. C. Watts, T. R. Wood, E. C. Hermann, and C. E. Hoffman. 1964. Anti-viral activity of R-adamantanamine (amantadine). *Science* 144:862-863.
2. Dawkins, A. T., L. R. Gallager, Y. Yogo, R. B. Hornick, and B. A. Harris. 1968. Studies on induced influenza in man. *J. Amer. Med. Ass.* 203:1095-1099.
3. Fallabella, F. 1967. Bleeding mice; a successful technique of cardiac puncture. *J. Lab. Clin. Med.* 70:981-982.
4. Francis, T. 1940. The inactivation of epidemic influenza virus by nasal secretions of human individuals. *Science* 91:198-199.
5. Grunnert, R. R., J. W. McGahen, and W. L. Davies. 1965. The in vivo anti-viral activity of 1-adamantanamine. *Virology* 26:262-269.
6. Jackson, G. G., R. L. Muldoon, and L. W. Akers. 1964. Serological evidence for prevention of influenza infection in volunteers by an anti-influenzal drug, adamantanamine hydrochloride. *Antimicrob. Ag. Chemother.* 1963 3:703-707.
7. McGahen, J. W., and C. E. Hoffman. 1968. Influenza infections in mice. *Proc. Soc. Exp. Biol. Med.* 129:678-681.
8. McGahen, J. W., E. M. Neumayer, R. R. Grunnert, and C. E. Hoffman. 1970. Influenza infections in mice. *N.Y. Acad. Sci.* 173:557-567.
9. Neumayer, E. M., R. F. Haff, and C. E. Hoffmann. 1965. Anti-viral activity of amantadine HCl in tissue culture. *Proc. Soc. Exp. Biol. Med.* 119:393-396.
10. Rabinovich, S., J. T. Baldini, and R. Bannister. 1969. Treatment of influenza. The therapeutic efficacy of rimantadine HCl in a naturally occurring influenza A₂ outbreak. *Amer. J. Med. Sci.* 267:328-335.
11. Schulman, J. L. 1968. Effect of 1-adamantanamine HCl and methyl-1-adamantanethylamine HCl in transmission of influenza virus infection in mice. *Proc. Soc. Exp. Biol. Med.* 128:1173-1178.
12. Togo, Y., R. B. Hornick, and A. T. Dawkins. 1968. Studies on induced influenza in man. *J. Amer. Med. Ass.* 203:1089-1094.