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Efficacy of rimantadine hydrochloride in the treatment of influenza infection of mice

John E. Herrmann, Matthew Bruns, Kim West and Francis A. Ennis

Division of Infectious Diseases, University of Massachusetts Medical School, Worcester, MA 01655, U.S.A.

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Summary

Rimantadine HCl was assessed for its effect on influenza A virus titer in lungs of infected BALB/c mice. Rimantadine administered orally via drinking water, with and without an intraperitoneal prophylactic loading dose, was compared to intraperitoneal administration. Mice were infected with a non-lethal dose of influenza A/Port Chalmers/H3N2 virus and the pulmonary virus titers were determined at intervals over a 21 day period. Prophylactic treatment with rimantadine followed by oral administration resulted in up to a 4 $\log_{10}$ reduction in pulmonary virus titer. The oral doses given to the mice were comparable on a mg/kg/day basis to those recommended for treatment of human infections. Reductions in pulmonary virus titers also occurred after intraperitoneal rimantadine treatment which included a prophylactic dose, but the reductions in pulmonary virus titers were less striking and not consistent over the course of infection. There were no significant reductions in pulmonary virus titers by either route if treatment was started 8 h after exposure to virus.

Influenza A virus; Rimantadine HCl

Introduction

Rimantadine is the alpha-methyl derivative of amantadine (l-adamantanamine) and has been shown to be effective against influenza A virus both in vitro and in vivo. The parent compound amantadine (which is the only anti-influenza drug that

Correspondence to: J.E. Herrmann, Division of Infectious Diseases, University of Massachusetts Medical School, Worcester, MA 01655, U.S.A.

is currently licensed for U.S. distribution) has been available for over 20 years, but has not been widely used primarily due to reports of central nervous system (CNS) side effects. Recently, there has been a renewed interest in rimantadine for treatment of influenza virus infection (Hall et al., 1987; Thompson et al., 1987) because it appears to be as effective an antiviral agent as amantadine, but does not appear to induce the same degree of CNS side effects associated with amantadine (Dolin et al., 1982). This difference may be related, in part, to the pharmacokinetics of the two drugs. It has been reported that plasma levels of amantadine 4 h after administration are about double that found with rimantadine at equivalent dosages (Hayden et al., 1983, 1985; Anderson et al., 1987). However, the 24–36 h half-life of rimantadine (Hayden et al., 1985, 1987; Wills et al., 1987) is approximately twice that of amantadine and the ratio of drug concentration in nasal mucus to the plasma drug concentration is higher for rimantadine than amantadine. These observations may explain the decreased frequency of CNS side effects without a decrease in clinical effectiveness of rimantadine (Hayden et al., 1985).

In human studies, oral administration of the drugs in one or two doses per day has been the regimen most often used to evaluate the efficacy of both amantadine and rimantadine. Administration of amantadine (Hayden et al., 1980) or rimantadine (Hayden et al., 1982) by aerosol has also been tested for the treatment of established influenza infections in attempts to improve therapeutic activity. Both drugs have been found to be highly effective in preventing influenza illness when given as prophylactic agents. They have been less effective in curing illness when used for therapy alone, but have been shown to reduce severity of symptoms and/or virus shedding (Hall et al., 1987; Thompson et al., 1987; Dolin et al., 1982).

In mice, the drugs have usually been administered by a parenteral route or by aerosol. Comparisons of these two routes of drug administration have concluded that the aerosol route is more effective (Stephen et al., 1975; Walker et al., 1976; Wilson et al., 1980). In one study, oral amantadine treatment given at specific intervals was not found to be as effective as aerosol therapy (Solovyov and Tolmacheva, 1967). These findings may be due, in part, to more efficient delivery of the aerosolized drug to the target tissue for virus infection, but may also be due to the continuous nature of the aerosol therapy. This may be especially important to maintain high plasma levels of rimantadine in mice. In contrast to the pharmacokinetics in humans, in whom the 24 to 36 h half-life of rimantadine is about twice that of amantadine, the 1.5 h half-life of rimantadine (Hoffman et al., 1988) in mice is less than the 2 h half-life of amantadine (Wood, 1965). Higher plasma levels are also obtained with equivalent single doses of amantadine in mice (Wood, 1965; Hoffman et al., 1988) as has been noted in humans, therefore more frequent treatment with rimantadine may be necessary for obtaining optimal antiviral activity in mice.

The delivery of antiviral agents by the aerosol route is cumbersome and thus is not likely to find widespread use in the treatment of human influenza virus infections, especially in adults. Previous studies utilizing administration of rimantadine via drinking water in high concentrations (approximately 75 times the comparable recommended human doses) have shown reductions in both the severity of disease

and the percent mortality in treated mice (McGahen et al., 1970). In this report, we have quantitated pulmonary virus titers over the course of non-lethal influenza infections in mice receiving oral treatment with rimantadine (in drinking water), compared to treatment by intraperitoneal administration. The oral doses of rimantadine used (mg/kg/day) were comparable to those recommended for treatment of human infections.

Materials and Methods

Mice

BALB/c male mice were used in these studies at 40 ± 2 days old and weighed approximately 20 g. Mice were housed in plastic microisolator cages, 6 to 9 mice per cage.

Virus and virus infection

Influenza A/Port Chalmers/H3N2 virus was used for infecting mice. Stock virus was prepared in 10 to 12 day old embryonated hen's eggs and the collected allantoic fluids were stored at −85°C. Virus was administered intranasally to ether-anaesthetized mice at 0.05 ml/mouse, 1×10^3 plaque-forming units (PFU) virus/ml.

Virus titration

Stock virus and mouse pulmonary virus were titrated in Madin-Darby canine kidney (MDCK) cell cultures by a modification of a plaque technique previously described (Tobita et al., 1975). For assay, MDCK cells were grown to confluency in 6-well tissue culture plates and rinsed with serum-free Eagle's minimal essential medium (MEM). Virus-containing samples were added (0.2 ml/well) in 0.01 M PBS, pH 7.0, containing 0.2% bovine serum albumin, and adsorbed for 1 h at 37°C. The cells were overlaid with MEM containing 1% Noble agar, 0.1% DEAE dextran, and 0.01 mg/ml trypsin (Sigma type III, Sigma Chemical Co., St. Louis, MO). After 3 days at 37°C, the cells were overlaid with 1% Noble agar containing 0.003% neutral red, incubated at 37°C, and the plaques read the following day.

To determine pulmonary virus content, mice were killed by injection of ketaset/xylazine, the lungs were removed aseptically, and placed in wells of a 24 well culture plate. Each well contained 2 ml MEM for rinsing the tissue. The lungs were homogenized in 2 ml MEM with a Dounce homogenizer. The mixture was clarified by centrifugation at 500 × *g* for 5 min, the pellet discarded, and the supernatant fluid stored at −85°C.

Administration of rimantadine

For intraperitoneal (i.p.) administration, rimantadine hydrochloride (Hoffmann-La Roche Inc., Nutley, NJ) was diluted in 0.01 M PBS, pH 7.0, at 84 mg per 10 ml PBS and filter-sterilized (0.22 μm diameter filter). This solution was given i.p. at 0.1 ml per 21 g mouse. For oral administration, rimantadine was added to distilled water (81 mg per 3 l), to give a concentration of 0.027 mg/ml. This was

given as drinking water for the mice to be tested, resulting in approximate dosages of 6 mg/kg/day based on the amount of water we determined was consumed by the mice (average of 4.5 ml/mouse/day).

Treatment regimens

In addition to the oral and i.p. routes of rimantadine administration, two general treatment regimens were used, one which included a prophylactic dose of rimantadine as part of the treatment, and one which involved post-infection therapy alone. For prophylaxis and treatment i.p., mice were injected with rimantadine (40 mg/kg) 30 min prior to infection with virus, and at 12, 24, and 36 h after infection. Six test mice (rimantadine treated) and 6 control mice (untreated) were used for each data point. Lungs were harvested for virus titration at days 2, 4, 7, 10, 14 and 21. To determine the efficacy of rimantadine given i.p. as a therapeutic agent post-infection, mice were infected with influenza virus and injected with 40 mg/kg rimantadine at 8, 32, 56, 80, 104 and 128 h after exposure to virus. Six treated mice and 4 control mice were analyzed for lung virus titers on days 2, 4, 6, 8, 10, 14 and 21 post-infection.

For oral treatment with prophylaxis, mice received an initial dose of rimantadine i.p. (40 mg/kg) 30 min prior to infection and per os via drinking water for the duration of the experiment (21 days). Six rimantadine-treated mice and 6 control mice were used for each data point. For therapy given post-infection only, test mice received one injection of rimantadine i.p. (40 mg/kg) 8 h after exposure to virus and received drinking water containing rimantadine for the duration of the experiment. Six rimantadine-treated mice and 6 control mice were used for each data point. Pulmonary virus titers were determined at days 2, 4, 6–10, 12, 14 and 21 in each experiment.

Results

Intraperitoneal administration of rimantadine

The pulmonary virus titers of the infected groups of mice which received a prophylactic dose of rimantadine i.p. in addition to i.p. therapy post-infection, along with titers of virus in the lungs of the untreated, infected control mice, are shown in Fig. 1. A significant reduction ($P<0.05$) in virus titers was observed in the lungs of rimantadine treated mice only on day 2 after infection. As indicated by the standard error bars, there was greater intra-assay variation in pulmonary virus titers among mice in the rimantadine treated group than in the control group. The results of experiments in which mice received rimantadine as post-infection therapy without a prophylactic dose are shown in Fig. 2. The patterns of infection appear similar for both rimantadine treated and untreated mice, and there were no significant differences between the pulmonary virus titers of the two groups on any of the days tested.

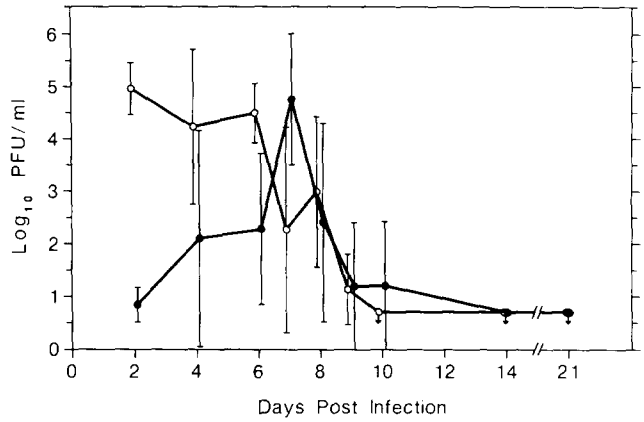

Fig. 1. Effect of rimantadine administered i.p. both prior to and after influenza virus inoculation. Symbols: (○), virus titers in lung tissue from untreated mice; (●), virus titers in lungs from rimantadine-treated mice. Arrows indicate virus concentration was below detectable levels. The results are means ± standard errors of samples from 6 mice for each time interval.

Oral administration of rimantadine

The results of experiments in which influenza virus-infected mice received a prophylactic dose of rimantadine i.p. prior to administration of virus, followed by continual oral therapy after infection, are shown in Fig. 3. There were significant reductions ($P<0.01$) in pulmonary virus titers between the rimantadine treated and untreated groups of mice on days 2, 4 and 6 after infection. The maximum decrease in the virus titer was 4.1 $\log_{10}$, detected on day 6. The intra-assay variations

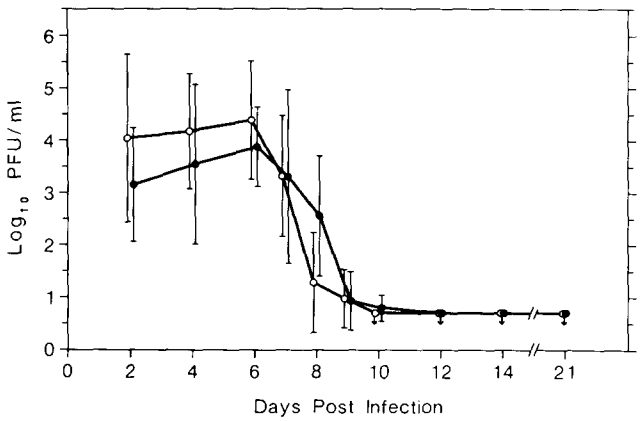

Fig. 2. Therapeutic activity of rimantadine administered i.p. after mice were infected with influenza virus. Symbols: (○), virus titers in lung tissue from untreated mice; (●), virus titers in lung tissue from mice treated with rimantadine. Arrows indicate virus concentration was below detectable levels. The results are means ± standard errors of samples from 6 rimantadine-treated mice and 4 untreated mice for each time interval.

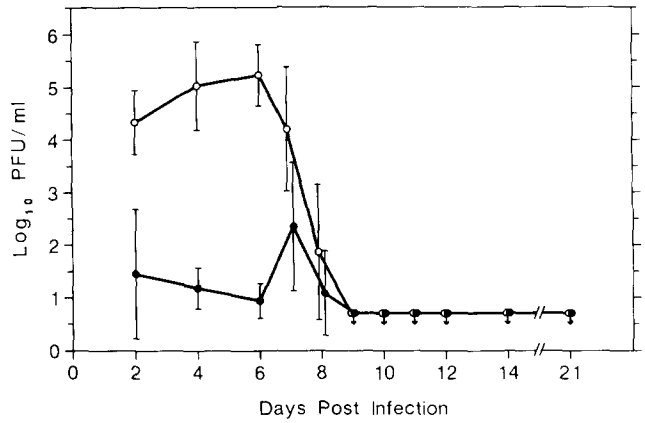

Fig. 3. Effect of continuous oral administration of rimantadine on influenza virus infection in mice. Rimantadine was given i.p. 30 min prior to infection, and orally via drinking water for 21 days. Symbols: (○), virus titers in lung tissue from untreated mice; (●), virus titers in lung tissue from mice treated with rimantadine. Arrows indicate virus concentration was below detectable levels. The results are means ± standard errors of samples from 6 mice for each time interval.

in pulmonary virus titers were similar in both the rimantadine treated and untreated groups of mice, as indicated by the standard error bars. Fig. 4 shows the results of experiments in which mice received rimantadine post-infection only. There was a marginally significant (P=0.057) reduction in pulmonary virus titer on day 6, and no significant differences on any of the other days tested.

Groups of infected mice which were rimantadine-treated or untreated both showed a net increase in weight (approximately 0.2 g per day) over the course of

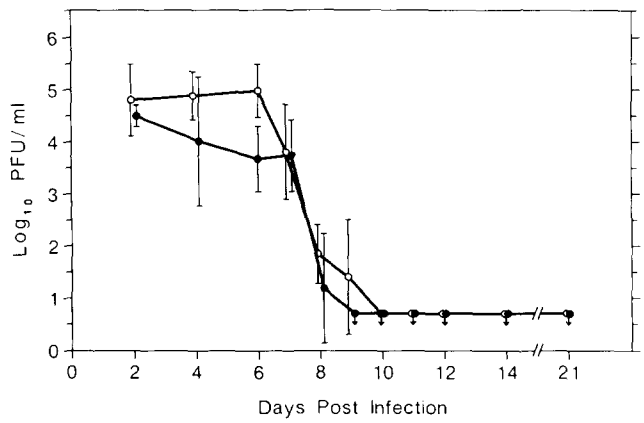

Fig. 4. Therapeutic activity of rimantadine given orally via drinking water after mice were infected with influenza virus. Symbols: (○), virus titers in lung tissue from untreated mice; (●), virus titers in lung tissue from mice treated with rimantadine. Arrows indicate virus concentration was below detectable levels. The results are means ± standard errors of samples from 6 mice for each time interval.

the experiments (data not shown). There were no significant differences in weight changes among the two groups for any of the treatment regimens.

Discussion

Studies concerning the efficacy of oral administration of either amantadine or rimantadine for prophylaxis and therapy of influenza in animal models have been limited, and the results of such studies have been variable. In ferrets, oral administration of amantadine the day following exposure to influenza virus exacerbated rather than alleviated the infection, as measured by lung damage and percent mortality (Cochran et al., 1965). In mice, prophylaxis with oral doses of amantadine or rimantadine has been reported to be effective in reducing severity of disease and percent mortality (Wood, 1965; Grunert et al., 1965). Oral doses given therapeutically have been reported to be effective in some cases (Grunert et al., 1965; McGahen et al., 1970), and in other cases less effective or ineffective (Solovyov and Tolmacheva, 1967; Grunert et al., 1965; McGahen and Hoffmann, 1968).

In these studies, we used a non-lethal dose of influenza virus and pulmonary virus titers were measured over the course of infection. Infection with the virus strain used did not cause a marked decrease in the weight of the mice that is seen with more virulent strains. Our results demonstrate some protective activity for rimantadine administered by the i.p. route as has been reported (Schulman, 1968). The times of i.p. administration and the doses given were selected on the basis of previous work (Schulman, 1968). It has also been reported that a single injection of amantadine before infection was as effective as a series of multiple doses starting before infection and continuing to 43 h after infection (Grunert et al., 1965). Thus, i.p. administration of rimantadine beyond the times we used did not appear to be warranted. However, we found the individual lung virus titers of mice treated with rimantadine i.p. mice to be highly variable in the experiments presented here and in others (data not shown). We also found that there was an increase in virus titers following cessation of treatment. A situation similar to this latter phenomenon has been seen in treatment of human infections, where it was found that children who had been treated with rimantadine for five days excreted virus for a longer period of time than those who had not been treated (Hall et al., 1987). We found no significant reduction in maximum lung virus titers after i.p. administration of rimantadine post-infection, which has also been previously reported (Stephen et al., 1975).

The most effective treatment regimen in our studies was found to be oral therapy following a prophylactic loading dose of rimantadine. The pulmonary virus titers among individual mice in the treated group were more consistent, and the $\log_{10}$ decrease in virus titer was higher than previously reported rimantadine treatment by any route. This was observed at doses of 6 mg/kg which are comparable to those which have been used for human treatment (6.6 mg/kg; Hall et al., 1987; Thompson et al., 1987) and were approximately 80-fold less than the doses previously used for peroral rimantadine treatment of mice (McGahen et al., 1970).

Administration of rimantadine by the oral route after virus inoculation was only marginally effective in reducing pulmonary virus titer. Aerosol therapy also has not been found to reduce maximum pulmonary virus titers in mice, although it has been found to increase the survival rate (Stephen et al., 1975; Walker et al., 1976). The reasons for the reduction in mortality rate without a corresponding reduction in peak pulmonary virus titers were not apparent. It was surmised that small delays in virus replication may affect increased survival, or that rimantadine has ameliorating effects such as improving functional capacity of treated lungs or may increase recovery from the pathological sequelae (Stephen et al., 1975).

These results confirm and extend earlier observations on the antiviral effects of rimantadine when given as prophylaxis and therapy for influenza A virus infections in the mouse model. We have shown significant and reproducible decreases in the lung virus titers of mice treated with rimantadine orally, after a single parenteral prophylactic dose. Administration of drug i.p. was far less effective. Thus, under these experimental conditions of a rather high challenge dose of virus that results in high titers of pulmonary virus and influenza pneumonia, we found rimantadine to have potent antiviral activity.

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