Effects of Small-Particle Aerosols of Rimantadine and Ribavirin on Arterial Blood pH and Gas Tensions and Lung Water Content of A2 Influenza-Infected Mice

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The respiratory pathophysiology of A2 influenza infection was studied in mice treated with small-particle aerosols (SPA) of rimantadine or ribavirin. Untreated infections in mice resulted in survival rates of 15% or less and were characterized by (i) severe hypoventilation (decreased P_O2 and increased P_CO2), (ii) compensated respiratory acidosis (increased P_CO2 and HCO3-, with normal pH), (iii) pneumonia with increased ratio of wet/dry lung weight, and (iv) hypothermia. Treatment with SPA of rimantadine (21 mg/kg per day for 4 days) beginning 72 h after virus challenge significantly improved survival rate (80%) but failed to alter lung pathology from that found in infected, untreated mice. Rimantadine treatment decreased somewhat the severity of hypoventilation, respiratory acidosis, lung wet weight, hypothermia, and lung virus titers from that observed in infected, untreated mice. SPA of ribavirin (26 mg/kg per day for 4 days) initiated 6 h after SPA exposure of mice to virus significantly improved survival rate (95%) and reduced lung virus titers and lung pathology. Gas exchange and pulmonary edema in ribavirin-treated, infected mice were significantly improved over those of infected, untreated controls. The mechanisms for increased survival rates induced by SPA of rimantadine remain uncertain, since increased survival rates could not be ascribed entirely to improvements in lung functions. In contrast, however, ribavirin treatment appeared to improve survival rates by reducing major lung pathology and pulmonary dysfunction. This was probably mediated through the antiviral effects of ribavirin.

Rimantadine hydrochloride (14, 18, 22) and ribavirin (7, 19, 20, 22) have been reported to be effective against influenza A infections, both in vitro and in vivo. Small-particle aerosols (SPA) of rimantadine used to treat influenza A infections of mice have markedly improved the percentage of survival without decreasing lung lesions. Oral ribavirin therapy of influenza A and B infections of mice resulted in marked reductions in lung virus titers and lung lesions and increased survival (7). The percentage of survival of ribavirin-treated, influenza A-infected mice was increased when the drug was given by SPA beginning 6 h postinfection (19, 22). In comparison to earlier treatment, survival was decreased when ribavirin therapy by either the intraperitoneal or aerosol route was delayed until 72 h postinfection (19, 22).

This report describes arterial blood pH and gas tensions and lung water contents of (i) A2 influenza-infected and noninfected mice, (ii) infected mice treated with SPA of rimantadine, and (iii) infected mice treated with SPA of ribavirin. Physiological evidence is provided which suggests that rimantadine and ribavirin increase survival rates of infected, treated mice, possibly by different mechanisms.

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MATERIALS AND METHODS

Animals and procedures. Seven-week-old female mice, Tac:(SW) FBR, were used in all studies. At periodic intervals after infection, five to eight mice per group were selected at random and weighed. Mice were anesthetized in a chamber through which flowed 1% halothane in room air at a rate of 2 liters/min. Blood was collected from the anterior abdominal aorta into dry, heparinized syringes. Rectal temperatures of mice were recorded before anesthesia and again immediately prior to blood sampling; the latter was used for temperature correction of pH values. The percentage of survival was calculated at 14 days from data on separate groups of mice, which were infected and treated simultaneously with all other experimental groups.

Virus. The mouse-adapted A/Aichi/2/68 (H3N2) strain of influenza virus was given to mice as an SPA with a mass median diameter of 2.2 μm, as
previously described (18). Preparation of inocula, sampling and titration of the exposure cloud, lung virus titers, lung lesion scores, and histopathology were accomplished as previously reported (18, 19, 22). The exposure dose of virus in all experiments was between 10^6.8 and 10^6.9 egg median infective doses, an approximate 90% lethal dose.

Drug. Rimantadine (α-methyl-1-adamantanemethylamine hydrochloride, E. I. DuPont de Nemours and Co., Inc., Newark, N.J.) and ribavirin (Nucleic Acid Research Institute of ICN Pharmaceuticals, Inc., Irvine, Calif.) were dissolved in sterile, triple-distilled water and given as SPA, as previously described (18, 19, 22). Presented doses were 21 and 26 mg/kg per day for rimantadine and ribavirin, respectively.

Blood gas tension. Arterial blood samples were analyzed within 10 min of collection for partial pressure of oxygen (Po2) and of carbon dioxide (PCO2) and pH. All determinations were performed using an automated analyzer (model 165, Corning Instruments, Inc., Medfield, Mass.), calibrated at 37°C. Algorithms of Ruiz et al. (16) were used to correct pH values to body temperature and to calculate bicarbonate.

Lung water content. Immediately after the blood sampling, the thorax was opened and all lung tissue was removed, weighed, and dried to constant weight in a vacuum oven. The ratio of wet/dry lung weight was calculated.

Rimantadine studies. Approximately 1,300 mice were used in duplicate experiments. Mice were allocated at random into one of three groups: uninfected-untreated (uninfected controls), infected-untreated (untreated), and infected-treated (treated). Rimantadine therapy was initiated 72 h after infection and given as a continuous SPA for 22 h/day for 4 days (19, 22). Three mice were selected at random on day 7 to verify lung virus titers and lung lesion scores.

Ribavirin studies. Approximately 350 mice for each of two experiments were allocated at random into one of three groups, as for the rimantadine studies. Therapy was initiated at 6 and 72 h postinfection in the two respective experiments. Drug was administered as an SPA for 80 min at the same time of day on 4 consecutive days (19, 22). Lung virus titers were determined, and lung lesions were scored on day 7. In both experiments, infected mice were sham-treated with distilled water (drug vehicle).

Drug controls. As a separate experiment, three groups of 40 uninfected mice each were given SPA of rimantadine (22 h/day for 4 days), ribavirin (80 min/day for 4 days), or distilled water (80 min/day for 4 days) as an SPA.

Statistics. Treatment groups were compared using one-way analysis of variance. Differences were considered significant when P < 0.05. Data from replicate experiments were pooled after it was determined by a two-way analysis of variance that there were no marked differences between experiments.

RESULTS

Influenza treated with rimantadine. Survival was 80% versus 7% in the respective treated and untreated groups, whereas lung virus titers were approximately 1 log_{10} lower in the former than in the latter (Fig. 1A). All deaths occurred between 4 and 9 days postinfection in both groups of mice, with the majority between days 6 and 8 (Fig. 1A). No significant differences were observed between untreated and treated mice in lung lesion scores (Fig. 1A).

Arterial PO2 values decreased in both untreated and treated mice and reached minimum values on days 8 and 6, respectively (Fig. 2A). Subsequently, PO2 values increased in surviving mice from both groups; however, between 7 and 14 days postinfection, values for treated mice increased earlier and were significantly closer to normal base line values than PO2 values of untreated mice (Fig. 2A). PCO2 values increased in both untreated and treated mice in parallel with the decrease in PO2 (Fig. 2B). The PCO2 values for treated mice were significantly closer to base line values than values for untreated mice on days 5, 6, 10, and 12.

Blood pH values were not significantly different among uninfected controls and the treated and untreated mice except for day 12 postinfec-

![Fig. 1. Effect of SPA of rimantadine or ribavirin on percentage of survival, lung virus titers, and lung lesion score of A2 influenza-infected mice. Rimantadine was administered for 4 days beginning 72 h postinfection. Ribavirin was administered for 4 days beginning at either 6 or 72 h postinfection. Symbols: (Δ) uninfected controls; (○) untreated; (●) treated.](http://aac.asm.org/Downloaded from http://aac.asm.org/ on October 23, 2017 by guest)
Rimantadine treatment of uninfected control mice caused no significant changes in \( P_{O_2} \), \( P_{CO_2} \), pH, \( HCO_3^- \), rectal temperature, lung water content, or body weight when compared to values for uninfected control mice.

Influenza treated with ribavirin. Ribavirin therapy initiated at 6 h postinfection resulted in lower lung lesion scores and lung virus titers when compared with untreated mice (Fig. 1B). Survival rates were 5 and 95% in untreated and treated mice, respectively (Fig. 1B). By contrast, ribavirin therapy initiated at 72 h postinfection, when untreated mice were significantly more acidic than treated mice (Fig. 2C). Blood bicarbonate values were maximum in untreated and treated mice on day 5 or 6 postinfection and subsequently decreased to base line values (Fig. 2D). Rectal temperatures decreased through day 6 in the two infected groups; however, the magnitude of the depression was significantly less in treated mice. Rectal temperatures in both groups of mice were close to base line values by day 19 (Fig. 2E). Wet/dry lung weight ratios increased after infection in both untreated and treated mice and reached respective maximum values on days 7 and 6 (Fig. 2F). Subsequently, in recovering mice, lung water content decreased in both groups, but remained elevated above values of uninfected controls (Fig. 2F). Body weights of both treated and untreated mice decreased through day 6. This weight loss continued through day 8 in untreated mice, but not in treated mice. The latter were significantly heavier by day 7 postinfection (Fig. 3A).
Infection resulted in no significant change in lung lesion scores or lung virus titers (Fig. 1C). When ribavirin therapy was initiated at 72 h, survival rates were 15% in treated mice and 10% in untreated mice (Fig. 1C).

Untreated, A2 influenza-infected mice showed no significant differences as compared to untreated mice in the previous rimantadine experiments (Fig. 2, 4, and 5). In contrast, ribavirin therapy initiated at 6 h postinfection produced significant physiological changes when compared with untreated mice (Fig. 4A–F). Arterial $P_{\text{O}_2}$ and $P_{\text{CO}_2}$ values of treated mice were significantly closer to base line values when compared with untreated mice throughout most of the experimental period (Fig. 4A and B). Arterial blood pH values were significantly different between the two infected groups of mice on day 8 postinfection, when treated mice developed an acidosis (Fig. 4C). Blood bicarbonate values were unchanged from normal base line values in treated mice in contrast to their significant elevation in untreated mice by day 5 (Fig. 4D). Rectal temperatures of treated mice remained normal, whereas untreated mice exhibited significant hypothermia throughout the experimental period (Fig. 4C). Ribavirin treatment initiated 6 h postinfection markedly diminished the severe pneumonia seen in untreated mice (Fig. 4F). Infected, ribavirin-treated mice showed significantly less weight loss than untreated mice by day 7 and weighed more than control mice by day 12 postinfection (Fig. 3B).

When ribavirin therapy was initiated 72 h postinfection, the effectiveness of treatment was markedly diminished (Fig. 5A through F) when compared with treatment initiated at 6 h postinfection. In general, $P_{\text{O}_2}$ and $P_{\text{CO}_2}$ values of recovering treated mice still returned to normal base line values significantly faster than values in untreated mice (Fig. 5A and B). Arterial blood pH and $HCO_3^-$ values of both groups of infected mice were not different throughout the experimental period (Fig. 5C and D). Significantly less severe hypothermia (Fig. 5E) and lung wet weight (Fig. 5F) were noted in recovering treated mice than in untreated mice. Both infected groups exhibited significant weight loss compared to controls during the experimental period (Fig. 3C). Treatment of uninfected control mice with ribavirin caused no significant changes in $P_{\text{O}_2}$, $P_{\text{CO}_2}$, pH $HCO_3^-$, rectal temperature, or lung water contents when compared to values for uninfected, untreated controls.

**DISCUSSION**

Experimental A2 influenza in laboratory mice is characterized by (i) severe hypoxemia (decreased $P_{\text{O}_2}$), (ii) inadequate alveolar ventilation (increased $P_{\text{CO}_2}$), (iii) compensated respiratory acidosis (normal blood pH and increased $P_{\text{CO}_2}$ and $HCO_3^-$), (iv) increased lung wet weight, (v) hypothermia, (vi) weight loss, and (vii) high mortality 5 to 9 days postinfection. Significant physiological changes in infected-untreated mice generally occur several days subsequent to peak lung virus titers and lung lesions (11, 18). Data accumulated on percentage of survival, lung virus titers, and lung lesions confirm the high morbidity and mortality caused by this disease in laboratory mice.
significant improvement in pulmonary function was observed until late in the course of the infection, and mortality remained elevated. It has been reported that peak lung virus titers are reached 48 h postinfection (11) and that by 72 h, bronchopneumonia is well established (19, 22). Therefore, the prevention of severe pathophysiological change in the early treated group can reasonably be attributed to the antiviral properties of ribavirin (19). When ribavirin therapy was delayed until after development of bronchopneumonia and peak lung virus titers, the drug was clearly less effective in preventing pulmonary dysfunction and mortality.

Clinical trials with ribavirin in humans against naturally acquired or artificially induced infection with influenza A virus show somewhat contradictory results (4, 17). Therapeutic ribavirin treatment was apparently effective in reducing clinical manifestations of naturally acquired influenza in young females (4). In contrast, others report that prophylactically administered ribavirin failed to alter development of signs and symptoms of artificially induced type A influenza illness in healthy male volunteers (17). Further, this drug was apparently only marginally effective against artificially induced influenza B illness in humans (21). The conclusion that ribavirin is effective in vitro (20, 21) and in mice (7, 19, 22), but not against experimentally induced infections in humans (17, 21), is enigmatic and may be premature. It is anticipated that additional studies with ribavirin in humans, some of which are in progress by others, will offer some resolution of this question.

When SPAs of rimantadine were administered 72 h postinfection, less severe pathophysiological changes were observed late in the course of infection. The rimantadine-treated mice recovered earlier than infected-untreated controls and had significantly improved survival rates. The slight but significant changes in $P_{O_2}$, $P_{CO_2}$, wet/dry lung weight ratios, and wet/dry lung weight ratios with SPAs of rimantadine-treatment may be very important in improving survival rates and speeding recovery of the infected, rimantadine-treated host. This is particularly noteworthy in view of the absence of any detectable lessening of lung lesions and only a slight reduction in lung virus titers of treated mice. A reduction greater than 1 log$_{10}$ in lung virus titers was also observed by Stephen et al. (18) in rimantadine-treated mice, which may be all that is necessary to improve survival rates significantly. It should also be noted that a direct comparison of the results with rimantadine to those of ribavirin was avoided in this study.

(18, 19, 22). Mortality in groups of infected mice treated at 72 h postinfection with SPA of ribavirin was higher than reported previously (19, 22). This difference could be an artifact due to the small number of mice (20 per group) set aside for determination of survival, a defect in the dissemination of ribavirin to treated mice, or a difference in animal susceptibility to the virus.

Ribavirin therapy initiated 6 h postinfection prevented the pathophysiological changes seen in infected-untreated mice and significantly increased percentage of survival. When ribavirin therapy was delayed until 72 h postinfection, no
because presented dosages of both drugs and treatment schedules (22 h/day with the former versus 80 min/day with the latter) were not identical for the two compounds.

In infected-untreated mice, $P_{CO_2}$ increased at the same time as hypoxemia developed (Fig. 2A and B). This type of response is characteristic of either severe, restrictive lung disease or depression of the carbon dioxide-sensitive chemoreceptors in the brain (2). In humans, influenza infections are associated with restrictive lung disease, with hypercapnia occurring after a period of hypoxia (5). However, central nervous system (CNS) involvement in primary influenza pneumonia has been reported (13). When mice are inoculated intracerebrally with non-neuroadapted type A influenza virus, CNS-type death occurs 5 to 7 days later (15). Treatment of type A influenza infection of mice with 85 or 100% oxygen decreases survival (1). In humans, oxygen therapy of influenza infection may result in reduced ventilatory drive (8). This finding in humans and increased deaths of mice (1) suggest that the hypoxic stimulus to ventilation via the carotid and aortic chemoreceptors may assume major importance, possibly due to impaired function of the carbon dioxide-sensitive chemoreceptors and associated mechanisms in the CNS.

Both rimantadine hydrochloride (14, 18, 22) and its structural analog amantadine hydrochloride (4, 6, 9, 12) are active in vivo against several strains of influenza virus. However, the efficacy of both drugs is apparently limited to type A influenza virus infection (9, 14, 22). Treatment of natural influenza A infection in humans with amantadine is associated with a shortened course of clinical illness and a more rapid resolution of the small airway dysfunction, with no effect on virus shedding or development of antibody titer (9, 12). Prophylactic amantadine treatment of artificially induced influenza A virus infection in humans yields less virus in nasal washings and reduced serum antibody titers and febrile responses (4). In addition, amantadine is an effective agent for the treatment of functional disturbance of the CNS, such as occurs in patients with Parkinson's disease, and has been shown to have an effect on central thermoregulatory control mechanisms of mice (3, 10). If we assume that rimantadine produces central effects similar to amantadine, then drug effects on the CNS may also partially explain improved survival rates in groups of rimantadine-treated, A2 influenza-infected mice. Further study will be necessary to implicate CNS function as an important facet of successful influenza therapy.

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LITERATURE CITED


