Therapeutic Efficacy of Rimantadine HCl in Experimentally Induced Influenza A Illness in Horses

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Oral treatment with rimantadine HCl[a] was used as a therapeutic medication to reduce the severity of experimental equine influenza infection. Authors' address: The Graduate Center for Toxicology and the Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546.

1. Introduction

Amantadine HCl and rimantadine HCl are effective in the prophylaxis of naturally occurring influenza A infections in humans.[1,2] Although both antivirals have similar therapeutic effects, rimantadine has fewer side effects associated with the central nervous system.[3]

We have previously reported on the pharmacology, pharmacokinetics, and neurological effects of amantadine and rimantadine.[4] This study was therefore designed to assess the antiviral efficacy and therapeutic benefit of rimantadine during an *in vivo* challenge infection of equine influenza in the horse.

2. Materials and Methods

A. Animals

The randomized, placebo-controlled clinical trial was performed in eight yearling horses (300–350 kg). These animals had negative (<10) hemagglutination-inhibition (HI) titers in trypsin-periodate-treated serum to a variety of equine 1 and equine 2 viruses, including KY/91 influenza virus. Rimantadine HCl was dissolved in ethanol:water (1:8) and administered in liquid form. The daily dose was 30 mg/kg q 12 h PO (08:00 and 20:00 h) via stomach tube. Plasma samples were taken to determine the rimantadine plasma concentration in each treatment horse.

B. Infection of Horses

The eight horses were inoculated by nebulization (DeVilbis) on day 0 with 1.0×10^6 EID_{50} of influenza A–equine 2–KY/91 virus in 5 ml PBS.

Serum samples were collected on days 0, 1, 2, 3, 4, 10, 14, and 21. A complete physical examination was performed each morning prior to dosing and consisted of a measurement of rectal temperature, heart rate, respiratory rate, and capillary refill time, and assessment of lymph nodes (submandibular and parotid) and lung and gastric motility sounds. Complete blood counts and serum chemistry profiles were performed prior to infection and again on day 4. Rectal temperatures were measured daily with a standard rectal thermometer. Significance was

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determined by the student t test for independent samples and the Friedman test, using a nonparametric randomized block design.

Nasopharyngeal swabs for virological testing were collected daily by using 2 × 2 in. (~5 × 5 cm) gauze held in a 20-in. (~50 cm) stainless-steel wire loop encased in rubber tubing. The swab was passed via the ventral nasal meatus up to the animal's nasopharynx approximately 25 cm to acquire the sample. The gauze was removed with sterile forceps and placed in transport medium containing PBS, glycerol, and antibiotics.[5]

C. Additional Medications

After the clinical examinations were completed, all animals were sedated by using a combination of detomidine and butorphanol intravenously to protect both horse and researcher during the nasogastric intubation.

D. Evaluation of Therapeutic Effect

Virus isolation and virus titers (EID_{50} units) from nasal swabs were determined by an injection of nasal swab samples into the allantoic cavity of 10-day-old embryonated chicken eggs followed by incubation at 35°C for 3 days, chilling to 4°C, then sampling the allantoic fluid for viral hemagglutinating activity. HI titers were performed on serum samples taken at the above time points to document viral infection and determine seroconversion to influenza HA antigen. Infection was determined by virus isolation from each nasal swab and seroconversion. As a way to determine whether or not rimantadine had a therapeutic effect on the respiratory system of each animal, each horse was stethoscopically examined daily and assigned a lung grade number from 1 to 6 on each of the five major regions (three regions located dorsally and two ventrally) of both the right and left lungs; after this, each animal was given a grade number based on all lung fields for that day.

3. Results

Rimantadine treatment (30 mg/kg q 12 h PO) for 7 days (four horses) was associated with significant decreases in pyrexia and lung sounds, and clinically relevant decreases in respiratory rate and heart rate, compared with placebo-treated controls. Mean rimantadine peak and trough plasma concentrations (ng/ml ± standard error) were 485.4 ± 123 and 180 ± 13, respectively. Drug-resistant viruses were isolated in nasal swabs taken from horses receiving rimantadine during the trial. The confirmation of drug-resistance was performed by using polymerase chain reaction (PCR) and nucleic acid sequencing techniques. Virus shedding was reduced in the rimantadine recipients on day 2 of the experiment, and the total time to recovery was decreased in the treatment group compared with that of controls.

4. Discussion

Chemotherapy may be required to reduce the severity of an influenza outbreak in which a new virus strain has arisen or immunity is lacking. To this end, we tested the effectiveness of rimantadine 30 mg/kg q 12 h in the face of an actual influenza infection. We examined and compared the mean rectal temperatures and detected a significant difference ($p < 0.05$) on day 2 between the treatment and control groups. Rectal temperatures normally reach a maximum on day 2 during a challenge period and often will reach another maximum if a secondary bacterial infection ensues.

When we examined the amounts of virus detected in swabs on day 2, we found that the drug treatment group had a reduced viral titer compared with controls, although drug-resistant mutant viruses were detected in the treatment group. This suggests that rimantadine had an effect on the replicating viral load in the treatment animals. We therefore consider rimantadine to be effective in reducing clinical signs, and if given early enough, this medication may effectively prevent virus replication during natural exposure.

References and Footnotes

1. Wintermeyer SM, Nahata MC. Rimantadine: a clinical perspective. *Ann Pharmacother* 1995;29:299–310.
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4. Rees WA, Harkins JD, Woods WE, et al. Chemotherapy of equine influenza: overview of the pharmacokinetics and neurological effects in the horse, in *Proceedings*. 11th Int Conf Racing Anal Vet 1996.
5. Chambers TM, Holland RE, Lai ACK. Equine influenza: current veterinary perspectives, part 1. *Equine Pract* 1995;17:19–23.

[a]Rimantadine is currently only available for human use. The manufacturer does not have an equine preparation for this compound at this time.