Pharmacokinetics and therapeutic efficacy of rimantadine in horses experimentally infected with influenza virus A2

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Objective—To determine pharmacokinetics of single and multiple doses of rimantadine hydrochloride in horses and to evaluate prophylactic efficacy of rimantadine in influenza virus-infected horses.

Animals—5 clinically normal horses and 8 horses seronegative to influenza A.

Procedure—Horses were given rimantadine (7 mg/kg of body weight, IV, once; 15 mg/kg, PO, once; 30 mg/kg, PO, once; and 30 mg/kg, PO, q 12 h for 4 days) to determine disposition kinetics. Efficacy in induced infections was determined in horses seronegative to influenza virus A2. Rimantadine was administered (30 mg/kg, PO, q 12 h for 7 days) beginning 12 hours before challenge-exposure to the virus.

Results—Estimated mean peak plasma concentration of rimantadine after IV administration was 2.0 µg/ml, volume of distribution (mean ± SD) at steady-state (Vd_{ss}) was 7.1 ± 1.7 L/kg, plasma clearance after IV administration was 51 ± 7 ml/min/kg, and β-phase half-life was 2.0 ± 0.4 hours. Oral administration of 15 mg of rimantadine/kg yielded peak plasma concentrations of < 50 ng/ml after 3 hours; a single oral administration of 30 mg/kg yielded mean peak plasma concentrations of 500 ng/ml with mean bioavailability (F) of 25%, β-phase half-life of 2.2 ± 0.3 hours, and clearance of 340 ± 255 ml/min/kg. Multiple doses of rimantadine provided steady-state concentrations in plasma with peak and trough concentrations (mean ± SEM) of 811 ± 97 and 161 ± 12 ng/ml, respectively. Rimantadine used prophylactically for induced influenza virus A2 infection was associated with significant decreases in rectal temperature and lung sounds.

Conclusions and Clinical Relevance—Oral administration of rimantadine to horses can safely ameliorate clinical signs of influenza virus infection. (*Am J Vet Med* 1999;60:888–894)

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Influenza is an acute and highly contagious disease of the respiratory tract in horses. Although specific horses may have generally mild disease, such infections can predispose horses to severe secondary bacterial infections.[1] Therefore, epizootics of influenza can cause considerable economic loss to horse owners. In specific herds, influenza is an important cause of morbidity and mortality in young and adult horses.[1] Vaccination is currently only partially effective in preventing the disease.[1] Veterinary practitioners have the capability for quick, stall-side confirmation of influenza in horses, using rapid diagnostic tests, which, in turn, would make antiviral prophylaxis or treatment with agents such as amantadine and rimantadine a viable option.[1] Until recently, these were the only drugs approved for use in treatment of humans with influenza. Therefore, we tested the potential for use of these drugs as anti-influenza agents in horses.

Rimantadine hydrochloride (α-methyl-1-adamantanemethylamine hydrochloride) is an aliphatic alkylated amine with pKa of 10.1 and molecular weight of 179. Its parent compound, amantadine, was discovered in the mid-1960s and found to have pharmacologic properties that included inhibition of influenza virus replication by blocking the viral M2 protein ion channel necessary for viral uncoating.[2-4] The rimantadine analog was developed to improve bioavailability of this agent and to reduce toxicosis associated with amantadine administration, which, in humans, includes nervousness, uncontrolled shaking, and sometimes seizures. Currently, use of rimantadine in humans is considered to cause fewer adverse effects than amantadine while having greater antiviral activity.[5-8]

Agents such as amantadine and rimantadine may cause adverse reactions in human patients during long-term treatment, and severity of these reactions may depend on the health status of the person. In humans, amantadine has been associated with more adverse effects of the CNS, compared with rimantadine, especially in patients with renal clearance of < 50 ml/min.[8,9] When administered orally to healthy subjects at the recommended dosage, both compounds caused adverse effects in the CNS and gastrointestinal tract, but the incidence of these effects was reportedly higher in patients taking amantadine, compared with patients taking rimantadine.[10-12]

To enable veterinarians to effectively use an agent in the prophylaxis and treatment of an infectious disease in horses, the therapeutic range of the agent must be defined, and dosing schedules must be developed that will yield safe and effective therapeutic plasma

(Table 3), compared with codon 136, and the major genotype differed among flocks. The overall allele frequencies for Q, R, and H at codon 171 were 57, 41, and 2%, respectively.

Discussion

In this study, we report development and validation of novel PCR-based methods to quickly genotype for the alleles of interest at codons 136 and 171 of the ovine *PrP* gene and document the feasibility of using buccal swab specimens as a source of DNA from sheep. The tests use small amounts of DNA, are easy to execute, require only basic molecular biology instrumentation, and can be completed within a few hours. Incorporation of a control restriction site further ensures that inaccuracies resulting from incomplete digestion will be eliminated and provides further quality control than that of PCR methods reported elsewhere. That all PCR analyses can be run at the same setting in a single machine should facilitate routine use in a laboratory setting.

The allele distribution at each codon is similar to those reported for Suffolk sheep of other studies.[14,17] Michigan is a major sheep-producing state, with Suffolks representing most of the purebred sheep population. Results of other studies[7,14,18] have indicated that presence of the R allele at codon 171 in homozygous or heterozygous animals confers increased resistance to scrapie, with the homozygous state being least risky. Similar to results of other studies using Suffolk sheep, the H allele was found to be rare in the Michigan Suffolk sheep examined. It was found in only 1 heterozygote and 1 homozygote sheep from flock 3. Furthermore, there is no evidence for the importance of the H allele vis-à-vis scrapie susceptibility in Suffolk sheep; thus, it may be reasonable to only test for the Q and R alleles using test 1.

Little variation in codon 136 of Suffolk sheep has been reported, and there is no strong evidence of relevance of codon 136 with respect to scrapie resistance in this breed. Therefore, we do not advocate routine evaluation of codon 136 alleles in Suffolk sheep. However, the V allele at codon 136 appears to correlate with resistance to scrapie in other breeds, such as the Cheviot,[6,12] Ile-de-France,[4] Romanov,[4] and Swaledale[15] breeds.

The variation in genotype frequencies among the 3 flocks evaluated is noteworthy. The owners and managers of flock 1, which had the lowest percentage of animals with the QQ genotype (10%), had been making a conscious effort to select for the R allele for the past 3 years; those of flock 2, working for the past 2 years, had 39% of their animals with the QQ genotype. The owners of the third flock, which had the highest percentage of animals with the QQ genotype (48%), had just begun to consider selecting for the R allele.

Genotype data should not be used to make inferences about the health status of individual sheep. The current state of knowledge indicates that sheep that carry susceptibility alleles will not develop scrapie in the absence of exposure to the scrapie agent.[19] Therefore, genotype data should be regarded as a guide to reduce risk of scrapie. The availability of simple, reliable, and affordable genotyping methods will serve to reduce the risk of scrapie in sheep flocks and, thus, improve the standing of the farmers who use these methods and increase the marketability of their products throughout the world. The ability to use a simple sample collection procedure, such as the buccal swab specimen collection method described here, should further facilitate genotyping efforts.

[a]Cytosoft, Medical Packaging Corp, Camarillo, Calif.
[b]Hoechst DNA Dye 33258, Amersham Pharmacia Biotech Inc, Piscataway, NJ.
[c]Hoeffer Model TKO 100 Spectrofluorometer, Amersham Pharmacia Biotech Inc, Piscataway, NJ.
[d]Robocycler, Stratagene Cloning Systems, La Jolla, Calif.
[e]ThermoSequenace, Amersham Life Sciences Inc, Cleveland, Ohio.
[f]QIAEX, QIAGEN Inc, Santa Clarita, Calif.

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concentrations. Furthermore, it must be determined whether the agent will provide useful protective effects against the specific infection in a clinical situation. We have reported in vitro antiviral efficacy, pharmacokinetic characteristics, and adverse reactions to amantadine in horses.[13] In the study reported here, we investigated pharmacokinetic variables and in vivo therapeutic efficacy of rimantadine in horses. Comparison of characteristics of these 2 antiviral agents will provide equine practitioners a clearer perspective on the risks and therapeutic benefits associated with use of these agents in horses.

Materials and Methods

Pharmacokinetic analysis

Horses and sample collection—Mature Thoroughbred mares weighing between 412 and 603 kg were used. These horses were in good health and maintained on pasture until the morning of the study. Horses were maintained on a regular seasonal deworming program and subjected to a complete examination (including CBC and serum biochemical analysis) by a veterinarian before and after treatments.

All oral administrations were performed by use of a nasogastric tube, and IV injections were administered rapidly into the left jugular vein. All blood samples were collected from the right jugular vein into evacuated tubes containing sodium heparin.[a] Plasma was separated by centrifugation and stored at −20 C until analyzed.

Protocol—An analytical method for rimantadine was developed, based on the method used for amantadine.[13] An initial study was performed to examine responses after IV administration of 5, 10, 15, or 20 mg of rimantadine[b]/kg of body weight. One horse was assigned to receive each of the aforementioned dosages to enable testing for adverse reactions.

Five horses were administered rimantadine in accordance with each of the following 3 regimens: 7 mg/kg, IV, once; 15 mg/kg, PO, once, without withdrawal of feed; and 30 mg/kg, PO, once, 14 hours after withdrawal of feed. Water was provided ad libitum during each phase. Rimantadine powder was dissolved in ethanol:water (1:8) and administered in liquid form. Plasma samples were obtained for chromatographic analyses 0, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, and 24 hours after IV administration and 1, 2, 3, 4, 6, 8, 10, and 12 hours after oral administration. Horses were allowed a 1-week interval between subsequent dosing regimens.

Pharmacokinetic characteristics for oral and IV administration of rimantadine in horses were determined, and mathematical projections of the plasma concentrations likely to be found after administration of multiple oral doses were developed, using pharmacokinetics derived from the data on oral and IV administration. To confirm the projections, 5 horses were administered rimantadine (30 mg/kg, PO, q 12 h for 4 days) to determine steady-state pharmacokinetics and disposition of the agent. Doses were administered at 8 AM and 8 PM each day. Hay was provided at 10 AM and 2 PM each day, and water was available ad libitum. Plasma samples were obtained at appropriate times to monitor peak and trough plasma concentrations of rimantadine.

Analytical methods—The extraction procedure was based on that developed for amantadine.[13] All chemicals and reagents used in the analysis of plasma samples were high-performance liquid chromatography-grade or better.

A gas chromatograph[c] equipped with a nitrogen-phosphorus detector and autosampler was used to analyze all extracts of the plasma samples for rimantadine.[13] A calibration curve constructed with standard concentrations of 25 to 1,000 ng/ml was used during each analysis.

Verification of the *tert*-butyldimethylsilyl[d] derivative of rimantadine was performed on several randomly selected plasma samples, using gas chromatography-mass selective detection.[e] Sample preparation and chromatographic conditions used were similar to those described by Herold et al.[14] The molecular ion of 293 MHz and a base peak at 158 MHz were used to characterize the rimantadine-*tert*-butyldimethylsilyl molecule.

Pharmacokinetic analyses were performed, using a nonlinear regression program.[f] **Area under the curve** (AUC) was measured by use of a linear trapezoidal approximation with extrapolation to infinity, and slope of the terminal portion of the log plasma rimantadine concentration versus time curve was determined by the method of least-squares regression.

Clearance after IV administration (**systemic clearance** [$CL_{systemic}$]) and after oral administration (**oral clearance** [CL_{oral}]) were calculated as follows:

$$CL_{systemic} = \frac{Dose^{IV}}{AUC^{IV}}$$

and

$$CL_{oral} = \frac{Dose^{PO}}{AUC^{PO}}$$

Steady state volume of distribution (Vd_{ss}) was calculated as follows:

$$Vd_{ss} = \frac{Dose \times \text{area under the moment curve}}{AUC^2}$$

Bioavailability (F) was calculated as follows:

$$F = \frac{AUC^{PO}}{AUC^{IV}} \times \frac{Dose^{IV}}{Dose^{PO}}$$

The term CL_{oral} was equivalent to $CL_{systemic}/F$, where F was the extent of bioavailability expressed as a fraction.

To project the effect of multiple doses on peak, trough, and mean steady-state concentrations, multiple-dose predictions were calculated for a hypothetical horse with the described mean absorption and disposition kinetics after oral and IV administration, using a commercially available computer program.[g]

Viral inoculation and therapeutic efficacy

Horses and sample collection—The randomized, controlled clinical trial was performed in a climate-controlled equine maintenance facility. Horses were housed in close proximity to each other in 3.3 × 4-m box stalls.

Yearling horses (300 to 350 kg) that were seronegative to KY/91 influenza virus, on the basis of results of hemagglutination inhibition tests, were used. Horses were fed 4 flakes of hay and grain once daily at 11 AM; water was provided ad libitum to ensure adequate drug absorption. Rimantadine was dissolved in ethanol:water (1:8) and administered (30 mg/kg, PO, q 12 h) in liquid form starting 12 hours before virus inoculation and continuing for 7 days. Medication was administered at 8 AM and 8 PM via nasogastric tube for treatment horses (n = 4), whereas control horses (4) received the same volume of solvent. As a preventative safety measure, all horses were administered a sedative (detomidine hydrochloride, 0.004 mg/kg; butorphanol tartrate, 0.002 mg/kg), IV, before nasogastric intubation. Plasma samples were obtained for determining the rimantadine plasma concentration in each treatment horse immediately before and 1 hour after administration of each dose during the treatment period.

Viral inoculation—All 8 horses were inoculated (day 0) by means of nebulization with 1.0×10^6 **egg-culture infectious dose 50%** ($ECID_{50}$) of field-isolated early-passage KY/91 influenza virus in 5 ml of phosphate-buffered saline (0.9% NaCl) solution.[1] Serum samples were obtained on days 0, 1, 2, 3, 4, 10, 14, and 21. A complete physical examination, consisting of rectal temperature, heart rate, and respiratory rate, was performed each morning before administration of rimantadine. Lungs were ausculted, and sounds were used to evaluate the therapeutic effect of rimantadine. Complete blood counts and serum biochemical analyses were performed immediately before and on day 4 of the study to evaluate short-term safety of rimantadine.

Nasopharyngeal mucosal swab specimens were collected daily, using 2 × 2-inch gauze sponges held in a 20-inch stainless-steel wire loop encased in rubber tubing. The swab was advanced into the horse's nose to a depth of approximately 30 cm to acquire the sample. The gauze was removed from the wire loop by means of sterile forceps and placed in a glass vial containing viral isolation media consisting of phosphate-buffered saline solution, glycerol, and antibiotics (penicillin, streptomycin, and gentamicin).

Virus isolation and titers (ie, $ECID_{50}$) were determined for nasal swab specimens, using 10-day-old embryonated chicken eggs. Titers for hemagglutination inhibition tests were determined on serum samples obtained at the aforementioned time points to document viral infection and determine seroconversion in accordance with international standards for serologic diagnosis of influenza in horses.[15]

Evaluation of infection—Infection was determined by means of viral isolation of nasal swab specimens and seroconversion based on results of hemagglutination inhibition assays. To determine whether rimantadine had a therapeutic effect, each horse was assigned a grade (0 to 4) for each of the 5 major regions of the right and left lung, determined by means of auscultation. Grades were assigned as follows: 0, sounds of a typical healthy lung; 1, minor inspiratory wheezes; 2, clearly audible inspiratory wheezes; 3, inspiratory and expiratory wheezes; and 4, sounds of mucus shunting (crackles) and severe wheezes. Lung grades were assigned once each morning, requiring approximately 15 min/horse. In addition, a dry cough, bilateral serous or mucopurulent nasal discharge, and lethargy were also signs of a flu-like illness but were not used in the scoring process.[16]

Statistical analysis—Mean rectal temperatures were compared by use of a repeated-measures test. Analysis of lung grades in the virus challenge-exposure study was performed by use of the Friedman test in a nonparametric randomized block design.[17] Rationale for use of that test was based on the ordinal nature of the data, which contrasts to interval or ratio data used for parametric analysis. Day of treatment was used as the blocking factor, because randomization was within day and the same horses were used throughout the study. Because the Friedman test was not designed to deal with replication within a treatment-block combination, the 8 horses were considered to be a treatment factor, and then a contrast was used to compare the control group to the treatment group. Horses were ranked within each day, 1 through 10. Days –3 through 0 were excluded from the analysis, because they did not contribute information to the analysis other than the fact that all horses had clear lung fields before the study. Next, the rank sum was computed for each horse, and test statistics were based on these rank sums. The test statistic to test for an overall difference between horses was as follows:

$$Q = 12/nk\,(k+1)\,(R_1^2 + R_k^2) - 3n\,(k+1)$$

where n is the number of blocks (ie, 10 days); and k is the number of horses in each block (ie, 8).

This test was used to detect evidence to conclude there was a difference between horses; however, a contrast was used to determine whether there was a difference between control and treatment groups.

An approximate z-test was used to compare rank sums for treatment and control horses. The contrast was in the following form:

$$C = (R_1 + R_2 + R_3 + R_4) - (R_5 + R_6 + R_7 + R_8)$$

which has a mean of zero and SD equal to $\sigma^2 = 2nk\,(k+1)/3$. The variance of this contrast was determined by use of the following equation:

$$\sigma^2 = Var(R_1 + R_2 + R_3 + R_4 - R_5 - R_6 - R_7 - R_8) = \Sigma^8_{i=1}\, Var(R_i) = 8\sigma^2 = 2nk\,(k+1)/3$$

where R_i is the rank sum for horse i.

Finally, a table of χ^2 values and a table of normal distribution values were used to determine P-values for this analysis. Significance was determined at a value of $P < 0.05$.

Results

Pharmacokinetic analysis

Adverse reactions—During the study reported here, we did not detect adverse reactions after a bolus injection at a rate of 5 mg/kg; however, clear-cut adverse reactions were observable immediately after administration of a dose at a rate of 10 mg/kg to 1 horse. These reactions included stumbling, incoordination, heavy breathing, nostril flaring, and apparent disorientation. Although apparent immediately after administration, these reactions subsided within about 20 minutes, and the horse made a full recovery. However, in another unrelated experiment, a horse died immediately after IV injection of rimantadine at a dosage of 5 mg/kg (data not shown).

Analytical methods—We developed a highly sensitive method of detecting rimantadine in equine plasma, using gas chromatography. Limit of quantitation of this method was 50 ng/ml, and the detector response was linear up to 100 ng/ml. Correlation coefficient for the standard curve was 0.998. Recovery of rimantadine from plasma samples was 60%, compared with 1,000% extraction from water, and range for the coefficient of variation of the standards for this analytical method was 6 to 9% after extraction from equine plasma. Mass spectral analysis confirmed that the material quantified in the plasma samples was indistinguishable from rimantadine.

Pharmacokinetic data—After rapid IV administration of rimantadine at a dosage of 7 mg/kg, plasma concentration of rimantadine best fit a two-compartment open model (**Fig 1**). Mean peak plasma concentration of rimantadine was slightly less than 2.0 µg/ml 5 minutes after administration and then decreased in a biexponential fashion. Plasma concentration of rimantadine was detectable for 6 hours after injection, after which it decreased to less than the limit of quantitation of the method. Analysis revealed that Vd_{ss} (mean ± SD) was 7.1 ± 1.7 L/kg, plasma clearance after IV administration was 51 ± 7 ml/min/kg, and the calculated β-phase half-life was 2.0 ± 0.4 hours.

After oral administration of a dose at the rate of 15 mg/kg, plasma concentration of rimantadine increased slowly and peaked between 1 and 2 hours

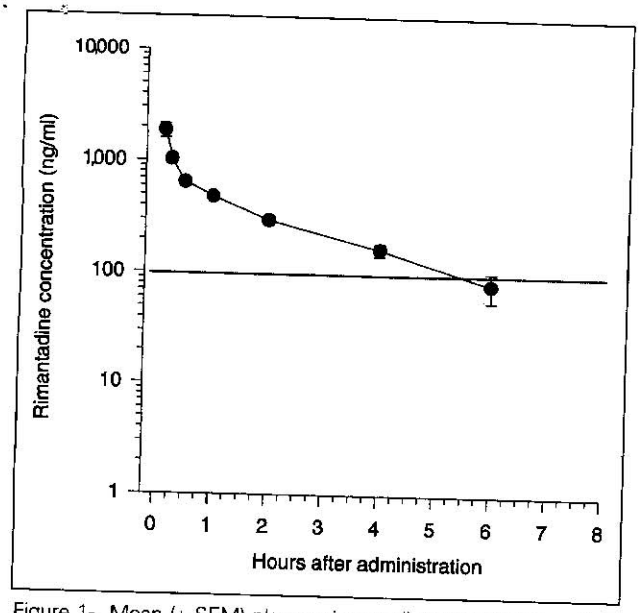

Figure 1—Mean (± SEM) plasma rimantadine concentrations in 5 Thoroughbred mares after a single IV administration of rimantadine (7 mg/kg of body weight). Horizontal line indicates the estimated minimum inhibitory concentration (100 ng/ml) for rimantadine.

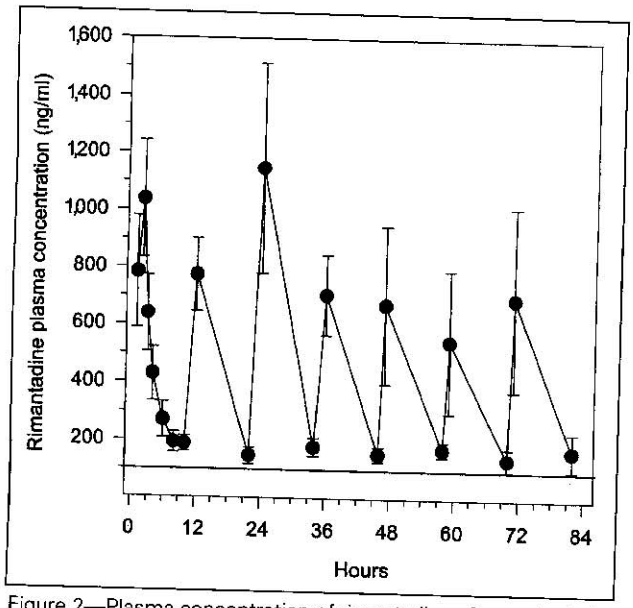

Figure 2—Plasma concentration of rimantadine after oral administration (30 mg/kg, q 12 h for 4 days) to 5 Thoroughbred mares. Horizontal line indicates the estimated minimum inhibitory concentration (100 ng/ml) for rimantadine. Time 0 = administration of initial dose.

after administration. Only 3 horses had detectable plasma concentrations of rimantadine, suggesting poor and variable oral bioavailability for this compound in horses (data not shown). Pharmacokinetic analyses of these data were not attempted.

Five horses were given rimantadine at a dosage of 30 mg/kg, PO, to increase the plasma concentration to greater than the estimated minimum therapeutic amount of 100 ng/ml. Plasma concentration of rimantadine peaked about 3 hours after administration and remained detectable in these horses for up to 8 hours (data not shown). Plasma clearance after oral administration was 340 ± 255 ml/min/kg, and the calculated β-phase half-life was 2.2 ± 0.3 hours. Bioavailability was calculated as 25% of the IV dose. This oral dose was well tolerated by all horses, and, with the exception of 1 horse, oral bioavailability of this agent at this dosage in these horses appeared relatively uniform.

Table 1—Mean antibody titer of control and rimantadine-treated horses to the KY/91 virus, as determined by results of hemagglutination inhibition assay. All horses were seronegative at the beginning of the study. Horses expressed antibody to KY/91 by day 10 after inoculation. Numbers represent the greatest dilution of trypsin-periodate-treated serum capable of completely inhibiting hemagglutination, using 8 hemagglutinating units of KY/91 virus. A negative sign (–) indicates a titer of < 1:10, which is the lower limit of detection. Day 0 = Day of inoculation

Day	Group	
	Control	Treatment
0	(–)	(–)
1	(–)	(–)
2	(–)	(–)
3	(–)	(–)
4	(–)	(–)
10	1:320	1:160
14	1:160	1:160
21	1:80	1:80

Pharmacokinetic simulation—To estimate a steady-state plasma concentration of rimantadine, pharmacokinetic variables generated from a single orally administered dose (30 mg/kg) were entered into a mathematical model to simulate plasma concentrations after repeated oral administration. These variables included clearance, half-life, bioavailability, and Vd_{ss}. Results of this simulation suggested that administration of 30 mg/kg, PO, q 12 h, would yield plasma concentrations greater than the minimum concentration of 100 ng/ml estimated to be required for therapeutic efficacy, yet less than the estimated toxicity threshold of 2 µg/ml.

Administration of multiple doses—To validate this proposed dosing schedule, 5 horses were administered multiple doses of rimantadine (30 mg/kg, PO, q 12 h for 4 days). Analysis revealed that this dosing schedule resulted in a mean plasma concentration of 519 ± 230 ng/ml with mean peak and trough concentrations of 811 ± 485 and 161 ± 60 ng/ml, respectively, thus confirming the pharmacokinetic projections (Fig 2).

Viral inoculation and therapeutic efficacy

Treatment confirmation—All 8 horses (4 rimantadine-treated and 4 control horses) used in the study reported here had laboratory confirmation of evidence of infection with KY/91 influenza A, as determined on the basis of virus shedding and seroconversion by day 10 (Table 1; Fig 3). None of the 4 rimantadine-treated horses had clinically detectable neurologic abnormalities while being maintained on the daily rimantadine regimen. Mean (± SEM) peak and trough plasma concentrations of rimantadine during the 7-day period were 485.4 ± 123 and 180 ± 13 ng/ml, respectively.

Viral shedding—Analysis of results for the nasal swab specimens inoculated into 10-day-old embryonated hen eggs revealed multiple days of viral shedding (Fig 3). All horses in treated and control groups shed virus dur-

ing this study. Control horses began shedding virus on day 1 of the study; however, rimantadine-treated horses appeared to have a delay in shedding, which was detected initially beginning on day 2. One horse in the treatment group continued to shed virus through day 7. The measured $ECID_{50}$ values (**Table 2**) indicated that control horses and treated horses shed similar amounts of virus on days 1 through 5 of the study.

Clinical signs—In the study reported here, our methods resulted in a moderate infection, comparable with those commonly observed in the field.[16] We did not identify significant changes in heart or respiratory rates between the 2 groups. However, significant differences were observed for rectal temperature and lung sounds between the control and treatment groups. In typical uncomplicated influenza infections in horses, fever generally is detectable on day 2 and is the first observable clinical sign. Rectal temperature in horses in the control group was significantly ($P < 0.05$) higher on day 2 of the study, compared with values for rimantadine-treated horses (**Fig 4**). Mean rectal temperature of the control group after day 2, although not significantly different from the treatment group, appeared to remain higher for a longer period, which contributed to extending this group's overall time to recovery. In this study, it appeared that the rimantadine-treated group did not have the classic fever spike commonly seen after an influenza viral infection.[16]

Other complications that may arise after an influenza infection often involve changes in lung sounds in severely affected horses. In this study, there was an increase in the overall lung grade in the control horses, compared with the rimantadine-treated horses, as determined on the basis of an experienced clinician

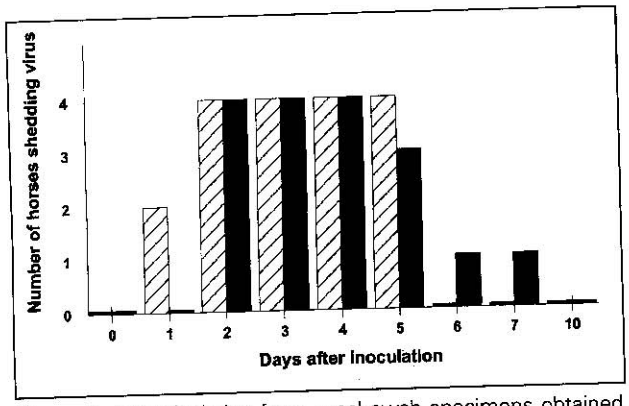

Figure 3—Virus isolation from nasal swab specimens obtained daily from 4 control and 4 rimantadine-treated horses. Isolation was performed by direct injection of swab contents into 10-day-old chicken eggs followed by analysis of the allantoic fluid, using a hemagglutination assay. ▨ = Control horses ■ = Rimantadine-treated horses.

Table 2—Viral shedding in control and rimantadine-treated horses. Values are mean log_{10} egg-culture infective dose 50% ($ECID_{50}$)/200 µl of fluid obtained from nasal swab specimens of horses inoculated with KY/91 influenza virus. Day 0 = Day of inoculation. Rimantadine-treated horses did not shed virus on day 1 of the study

	Day					
Group	0	1	2	3	4	5
Control	0.00	1.00	3.36	2.56	2.04	2.00
Treatment	0.00	0.00	2.82	2.07	2.69	1.15

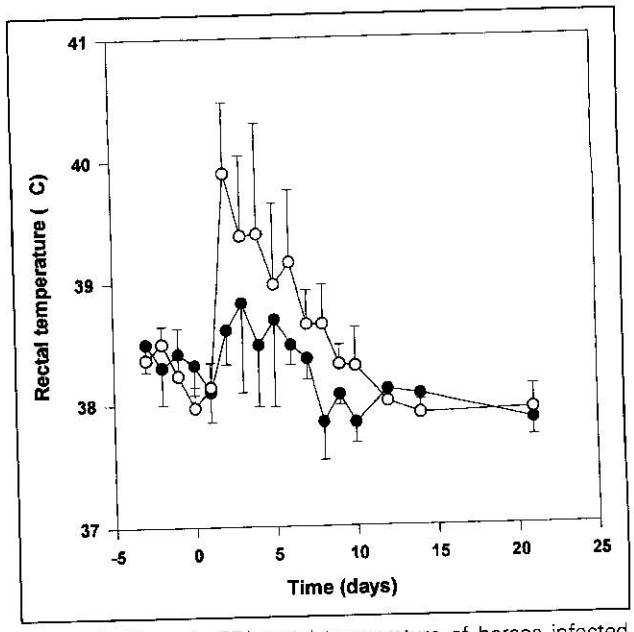

Figure 4—Mean (± SD) rectal temperature of horses infected with influenza virus. Day 0 = Day of inoculation. O = Control

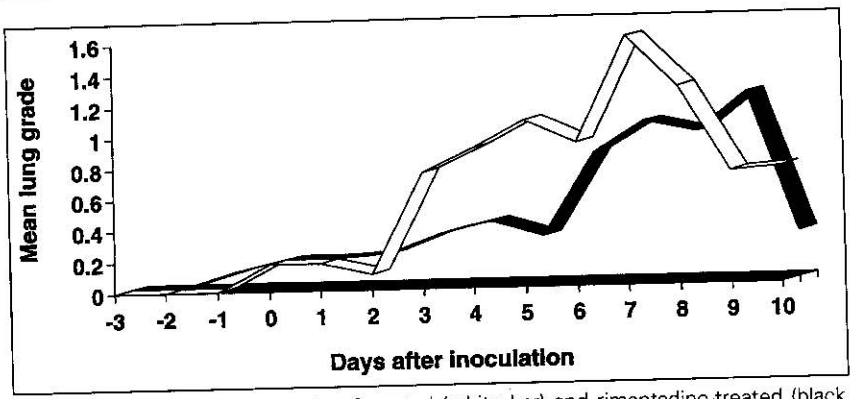

Figure 5—Mean daily lung grade of control (white bar) and rimantadine-treated (black bar) horses during a course of influenza infection. Day 0 = Day of inoculation. Statistical analysis, using the Friedman test, indicated that the 2 groups differed significantly ($P < 0.05$) over the entire treatment period.

(REH) who was responsible for evaluation of lung sounds. Not every lung quadrant was affected severely, however, resulting in mean lung grades of < 2. Mean lung grade for control and treated horses from days 0 through 10 of the study were determined (Fig 5). Analysis of the data by a nonparametric method revealed a significant ($P < 0.05$) difference in mean lung sounds between the groups for days 1 through 10.

Discussion

The study reported here was designed to determine the major attributes of rimantadine in regards to pharmacokinetics, disposition, and therapeutic efficacy in horses. To our knowledge, this is the first reported use of rimantadine in horses, and these results could be useful to veterinarians facing an outbreak of influenza in horses or when treating specific horses with the disease.

Sensitivity of influenza viruses to amantadine was documented initially by other researchers[5] and confirmed, using more recent virus isolates, by our research group.[13] For all isolates tested against amantadine and rimantadine, the antiviral efficacy of rimantadine was greater than that of amantadine. In fact, rimantadine was up to 10 times more effective than amantadine. On the basis of these and other data,[13] we estimated that it would require a minimum therapeutic concentration of 100 ng of rimantadine/ml be maintained in plasma to inhibit viral replication in horses. On the other hand, we estimated that peak plasma concentrations should not exceed 2,000 ng/ml to reduce the risk of acute CNS toxicosis or other adverse reactions. Adverse reactions to amantadine and rimantadine in humans commonly take the form of CNS adverse effects during long-term oral administration. We have reported neurotoxicosis associated with high plasma concentrations of amantadine in horses after IV administration.[13] On the basis of reports regarding adverse reactions of humans to rimantadine, we expected to see reduced toxicosis for rimantadine after IV administration. During the kinetic phase of the study, none of the horses developed seizure-like activity after rimantadine administration. In contrast to oral administration, it appeared that IV administration of rimantadine (similar to amantadine) can yield transiently high concentrations of this agent with a substantial risk for toxicosis and seizure activity. Therefore, for IV administration, rimantadine should only be administered slowly, at minimal dosages, and under carefully controlled conditions.

After oral administration of single and multiple doses of rimantadine, clinical signs or incidents of CNS distress were not observed in the horses of our study. However, when rimantadine was administered orally at a dosage of 15 mg/kg, differences in absorption among horses were apparent, and some horses did not maintain plasma concentrations of rimantadine that were greater than the proposed minimum inhibitory concentration for a useful period (ie, > 6 hours). Therefore, we increased the oral dosage to 30 mg/kg and documented that a single administration at this dosage yielded safe plasma concentrations of rimantadine that were greater than the proposed minimum inhibitory concentration for approximately 8 hours in 5 horses.

Computer simulations, using kinetic data obtained from a single oral administration (30 mg/kg), suggested that 30 mg/kg, PO, q 12 h, would maintain plasma concentrations of rimantadine sufficient to produce viral inhibition. In fact, when horses were given rimantadine at a dosage of 30 mg/kg, PO, q 12 h for 4 or 7 days, they maintained plasma concentrations greater than the therapeutic concentration of 100 ng/ml and did not have overt signs of toxicosis during the treatment period.

The in vivo study was used to test whether rimantadine would have an effect on influenza viral replication and clinical signs when used as a prophylactic treatment regimen. Our previous experiments suggested that 30 mg/kg of rimantadine HCL would provide the necessary minimum inhibitory steady-state concentrations in plasma and presumably in lung tissue. To test this, we chose the equine-2/KY/91 strain of influenza virus as our virus for challenge-exposure, using a dose of 10^6 $ECID_{50}$/horse on the basis of our knowledge about the virus strain's high infectivity rate.[1] It was established by Mumford et al[19] that full clinical signs of experimentally induced influenza infection only develop when ponies are exposed to a dose of 10^6 $ECID_{50}$/horse with the test virus A/equine/Newmarket/79.

Critical to the study was the development of clinical signs in our control group. It has been reported[18] that during clinical trials with rimantadine and amantadine in humans, the virus always replicates in treated patients, but reductions in viral load and clinical signs appear to validate usefulness of these medications. Therefore, efficacy in our study depended on a reduction of viral titers in nasal swab specimens as well as a reduction in clinical signs. When we compared mean rectal temperatures of the 2 groups, we found a significant ($P < 0.05$) difference on day 2 between the treatment and control groups. Rectal temperatures typically reach a maximum on day 2 after challenge-exposure and often will reach another maximum if a secondary bacterial infection ensues.[16] During our challenge-exposure, we did not observe a second increase in rectal temperature.

Our observations appear to be consistent with those of studies involving humans, who, upon receiving rimantadine to combat influenza infection, typically had a decrease in pyrexia, compared with those receiving a placebo. When we examined the $ECID_{50}$ virus titer of swab specimens obtained on day 2, we found that the treatment group had a lower viral titer, compared with the titer for the control horses, although these results were not significantly different. This suggested that rimantadine had an inhibiting effect on the replicating viral load in treated horses. When considering the amount of virus (10^6 $ECID_{50}$/horse) given during challenge-exposure, we would not expect to see a complete arrest of viral replication, but, rather, a reduction in viral titers.

The potential benefits associated with rimantadine use during an outbreak of influenza include prophylaxis and treatment of affected or exposed horses until an appropriate vaccine is administered. However, as expected for any antiviral drug, the use of rimantadine also

may generate drug-resistant mutant viruses. Humans with confirmed influenza infection who have been given rimantadine shed virus during each day of rimantadine treatment,[18] and even though they had substantial improvement with regards to their clinical signs, compared with those receiving a placebo, these treated patients had viruses that were resistant to rimantadine. Hence, potential veterinary application mirrors human medicine in that rimantadine use may be warranted to combat outbreaks of influenza despite the possible generation of resistant viruses.

The study reported here revealed that oral administration of 30 mg of rimantadine, q 12 h, produced safe plasma concentrations in 4 horses without causing clinically observable signs of toxicosis and effectively reduced clinical signs of influenza. To avoid adverse reactions associated with bolus delivery of rimantadine, IV administration should not be attempted.

[a]Vacutainer, Becton-Dickinson Corp, Rutherford, NJ.
[b]Rimantadine hydrochloride powder, Forest Pharmaceuticals, St Louis, Mo.
[c]Varian, St Louis, Mo.
[d]Pierce Chemical Co, Rockford, Ill.
[e]Hewlett Packard 6890 Series 3 MSD, Hewlett Packard, Palo Alto, Calif.
[f]RSTRIP, Micromath, Salt Lake City, Utah.
[g]SCIENTIST, Micromath, Salt Lake City, Utah.

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