

CHEMOTHERAPY FOR EQUINE INFLUENZA: OVERVIEW OF THE PHARMACOLOGY AND SIDE EFFECTS OF AMANTADINE AND RIMANTADINE IN THE HORSE

W. A. Rees^{*,†}, J. D. Harkins[†], W. E. Woods[†], R. A. Blouin^{*,**}, M. Lu^{**}, C. Fenger[†], R. E. Holland[†], T. Tobin[†], and T. M. Chambers[†]

^{*}The Graduate Center for Toxicology, [†]The Maxwell H. Gluck Equine Research Center;

^{**}The College of Pharmacy at the University of Kentucky, Lexington, Kentucky 40546, USA

ABSTRACT

The potential for effective chemoprophylactic or chemotherapeutic intervention in equine influenza has been enhanced by the advent of rapid diagnostic technologies. Therefore, we investigated the pharmacology in the horse of 2 anti-influenza medications, amantadine and rimantadine. *In vitro* studies indicate that both compounds suppress replication of several strains of equine-2 influenza virus. We developed methods for their analytical detection and investigated their bioavailability, disposition, and adverse reactions in horses. Both compounds are potentially effective for chemoprophylaxis in horses. However, they can cause unpredictable and potentially lethal seizures after iv administration, although no adverse reactions have been detected after oral administration.

INTRODUCTION

Influenza is a common acute equine respiratory disease. Vaccination has been only partly effective in its control due to a combination of inadequate quality controls, antigenic 'drift' of circulating virus strains and inherent limitations of the equine immune system. Clinical signs associated with influenza infections include fever, lethargy and increased lung sounds due to excess mucus production. The desquamation of the ciliated respiratory epithelium and damage to the mucociliary escalator function expose the lower respiratory tract to other invading particles; consequently infected horses should be stall-rested for 3 or more weeks to allow complete recovery of ciliary function.

Influenza itself is seldom life-threatening in horses but it predisposes them to secondary bacterial infections that can be. Also, it may predispose them to chronic respiratory tract abnormalities such as exercise induced pulmonary haemorrhage (EIPH). In an influenza outbreak, the disease spreads rapidly to

every susceptible in-contact horse; consequently influenza may be responsible for the suspension of racing operations at affected race tracks. Control of influenza in exposed racing horse populations is, therefore, of significant economic benefit.

Until recently it took upwards of 3 days, and a suitably equipped diagnostic laboratory, to confirm a diagnosis of equine influenza infection. This precluded effective veterinary intervention against the viral infection except for quarantine measures. However, diagnostic technologies have been developed that provide the capability for rapid confirmation of a diagnosis in the field. One such test is the Directigen FLU-A kit (Becton Dickinson Corp., Maryland, USA) which can detect equine influenza virus in nasopharyngeal swabs from naturally and experimentally infected horses (Chambers *et al.* 1994). The test is simple, self-contained, easy to interpret and requires only 15 min to obtain the result. This allows the veterinarian to institute rapid therapeutic intervention.

In man, amantadine hydrochloride has been approved by the US Food and Drug Administration for treatment and prophylaxis of all types of influenza infection since 1976. A related compound, rimantadine hydrochloride, was approved for influenza treatment and prophylaxis in adults, and prophylaxis in children, in 1993. These medications are used primarily in high risk populations including immunocompromised persons and elderly persons in nursing homes. Amantadine (1-adamantanamine) is an aliphatic, highly water-soluble polycyclic primary amine with a pKa of 10.1 and molecular weight of 151. The derivative rimantadine (α -methyl-1-adamantanemethylamine) is an alkylated amine with a pKa of about 10.1 and molecular weight of 179; it was developed to improve the bioavailability and reduce the toxicity associated with amantadine administration which includes nervousness, tremor and sometimes seizures (Hayden *et al.* 1983; Aoki *et al.* 1985; Anon 1994).

TABLE 1: Pharmacokinetic parameters of amantadine after administration of 10 mg/kg bwt iv and 20 mg/kg bwt orally (mean \pm sd)

Dose (mg/kg bwt)	Volume of distribution (l/kg)	$t_{1/2}$ (β -phase)	Clearance (ml/min/kg)	Bioavailability
iv 10	4.87 \pm 1.9	1.83 \pm 0.8	36.8 \pm 12.4	
Oral 20	—	3.37 \pm 1.4	185.2 \pm 183	60–40%

Bryans *et al.* (1966) first demonstrated that amantadine was effective in protecting horses against challenge with influenza virus. Rees *et al.* (1997) extended this to show that, based on *in vitro* testing, contemporary strains of equine-2 influenza virus (Equine/KY/91, KY/92, KY/93, KY/94, Miami/63) retained sensitivity to amantadine and also were sensitive to rimantadine. Thus these compounds, the first generation of anti-influenza chemo-therapeutic agents approved for clinical use, may also be useful for treatment/prophylaxis of equine influenza. We therefore evaluated the pharmacology and kinetics of these compounds to determine safe and effective dosing protocols in the horse. This report presents preliminary findings on the pharmacokinetics and adverse reactions to amantadine and rimantadine in the horse.

MATERIALS AND METHODS

Pharmacokinetic analyses were performed after iv (n=5) or oral (n=5) administration of amantadine or rimantadine to adult Thoroughbreds of 412–603 kg bwt. The horses were in good health, regularly dewormed, and kept on pasture except on days of experiments. They were given complete physical examinations including blood counts and serum chemistry profiles before and after all treatments.

Amantadine HCl was purchased from Aldrich Chemical Co., Missouri, USA. Rimantadine HCl was provided by Forest Pharmaceutical Co., Missouri, USA. Drugs were administered either by iv injection into the left jugular vein or oral intubation using a stomach tube. Plasma samples were taken from the right jugular vein using Vacutainer sodium heparin tubes (Becton Dickinson Corp., New Jersey, USA), separated by centrifugation and stored at -20°C until analysed.

For analysis, internal standards were added to plasma samples and test and standard compounds were extracted by using dichloroethane as solvent. All chemicals and reagents used were HPLC grade or better. Extracts were analysed for amantadine or rimantadine by using a gas chromatograph (Varian 3400, Missouri, USA) equipped with a nitrogen-phosphorus detector. Pharmacokinetic analyses were completed by using a non-linear regression program, RSTRIP (Micromath Inc., Utah, USA). Area

under the curve was estimated by linear trapezoidal approximation with extrapolation to infinity, and the terminal half life was determined from the slope of the semilog plot as estimated by the method of least squares regression.

Multiple dose predictions of peak, trough and mean steady-state plasma levels of the drugs were calculated using SCIENTIST (Micromath Inc.).

RESULTS AND DISCUSSION

Based on *in vitro* analyses of 50% cell culture infectious dose reduction, we estimated the typical minimal inhibitory concentrations of these drugs against different strains of equine influenza viruses to be 30–300 ng/ml for amantadine, and approximately 3- to 10-fold less for rimantadine. These are similar to the results of others using influenza viruses of different species including man. The trough plasma concentration of amantadine at steady-state achieved by established human therapeutic regimens for influenza is 300 ng/ml (Aoki *et al.* 1985). Our *in vitro* results suggest that effective dosing regimens for influenza treatment in horses should maintain trough concentrations of 300 ng/ml for amantadine or 100 ng/ml for rimantadine.

We have completed preliminary analyses of the pharmacology of amantadine and rimantadine. The pharmacokinetic parameters generated from iv and oral administration of amantadine are presented in Table 1. Preliminary work on the pharmacokinetics of rimantadine is now in progress; it appears that these 2 antivirals have similar plasma $t_{1/2}$ after iv administration, but their bioavailability, volume of distribution and clearance are different, which affects their disposition during multiple oral dosing.

Amantadine (10 mg/kg bwt) administered iv to horses produced detectable plasma concentrations (>50 ng/ml) for about 8 h. The mean $t_{1/2}$ of 1.8 h is much shorter than in man (16 h; Aoki *et al.* 1985), thus requiring more frequent dosing (bid or tid). When given orally via stomach tube (20 mg/kg bwt), variable plasma $t_{1/2}$ of 6–12 h post dose were obtained in different animals, and therapeutically effective plasma concentrations were not attained in 2 of 6 horses. Withholding feed for several hours before oral dosing did not affect the bioavailability of the drug significantly (40–60%).

High doses of either agent, especially when administered iv, can be acutely toxic in the horse. Amantadine causes central nervous system stimulation, represented as increased motor activity, tremors, anorexia, heightened sensitivity to external stimuli, emesis and convulsions (Vernier *et al.* 1969). In the horse, the LD₅₀ for orally administered amantadine is reported only as >96 mg/kg bwt (Vernier *et al.* 1969) and the oral LD₅₀ of rimantadine in the horse has not been established. Our preliminary studies suggest that the oral rimantadine treatment produces no short term ill effects. Peak plasma concentrations of amantadine >2,000–4,000 ng/ml appear to be associated with seizure, which is similar to plasma concentrations associated with acute side effects including seizure in man (Aoki *et al.* 1985). Thus iv administration of these drugs should be at a slow rate (eg <2 mg/kg/min) and should not be by bolus injection.

A potential problem with amantadine or rimantadine treatment for equine influenza is the development of viral resistance to these agents. This has been well documented in other animal models as well as in human clinical situations. Amantadine resistance is associated with particular amino acid substitutions in the influenza viral M2 protein (Hayden *et al.* 1991); thus current international surveillance efforts for equine influenza can be extended readily to detect incidence of resistant virus strains in circulation. In man, amantadine-resistant influenza viruses are no more virulent or transmissible than wild-type viruses, and the resistance phenotype does not appear to be maintained in the absence of the drug. Thus while amantadine or rimantadine should be used with care, the likelihood is that development of resistant viruses will be localised phenomena.

These data indicate that therapeutic levels of amantadine can be achieved in horses by iv or oral routes, and our work in this area with rimantadine is in progress. To date, the only actual equine influenza viral challenge of either drug in horses is the experiment of Bryans *et al.* (1966), which showed that oral administration of amantadine (20 mg/kg bwt) bid caused marked reduction in the duration of virus excretion, from mean 6 days to 1 day, in horses experimentally infected with equine-2 influenza virus 26 h after the first dose.

In summary, we explored the pharmacology of amantadine and started work with rimantadine as a prophylactic or therapeutic treatment for equine influenza. Both are effective inhibitors of equine influenza viruses *in vitro*, and dosing regimens that yield prophylactic and therapeutic concentrations of these agents in the horse are being developed.

ACKNOWLEDGEMENTS

This work was supported by the Grayson Jockey Research Foundation, Inc., the Royal Hong Kong Jockey Club, the National and Florida offices of the Horsemen's Benevolent and Protective Association, the Kentucky Racing Commission, the Kentucky Equine Drug Council, Mr. Paul Mellon and Mrs. John Hay Whitney.

Published as Kentucky Agricultural Experimental Station Manuscript #96-14-152 with the approval of the Dean and Director, College of Agriculture and Kentucky Agricultural Experimental Station. Publication #227 from the Equine Pharmacology and Experimental Therapeutics Laboratory, Department of Veterinary Science, University of Kentucky, Lexington, Kentucky.

REFERENCES

- Anon (1994) U.S. Department of Health and Human Services, Centers for Disease Control, Prevention and Control of Influenza: Part II, Antiviral Agents (1994). Morbidity and Mortality Weekly Report 34, no. RR-15.
- Aoki, F.Y., Stiver, H.G., Sitar, D.S., Boudreault, A. and Ogilvie, R.I. (1985) Prophylactic amantadine dose and plasma concentration-effect relationship in healthy adults. *Clin. Pharmacol. Therap.* **37**, 128-136.
- Bryans, J.T., Zent, W.W., Grunert, R.R. and Boughton, D.C. (1966) 1-adamantanamine hydrochloride prophylaxis for experimentally induced A/equine 2 influenza virus infection. *Nature* **212**, 1542-1544.
- Chambers, T.M., Shortridge, K.F., Li, P.H., Powell, D.G. and Watkins, K.L. (1994) Rapid diagnosis of equine influenza by the Directigen FLU-A enzyme immunoassay. *Vet. Rec.* **135**, 275-279.
- Hayden, F.G., Hoffman, H.F. and Spyker, D.A. (1983) Differences in side effects of amantadine hydrochloride and rimantadine hydrochloride in healthy adults. *Antimicrob. Agents Chemother.* **23**, 458-464.
- Hayden, F.G., Sperber, S.J., Belshe, R.B., Clover, R.D., Hay, A.J. and Pyke, S. (1991) Recovery of drug-resistant influenza A virus during therapeutic use of rimantadine. *Antimicrob. Agents and Chemother.* **35**, 1741-1747.
- Rees, W.A., Harkins, J.D., Woods, W.E., Blouin, R.A., Lu, M., Fenger, C., Holland, R.E., Chambers, T.M. and Tobin, T. (1997) Amantadine and equine influenza: pharmacology, pharmacokinetics and neurological effects in the horse. *Equine vet. J.* In press.
- Vernier, V.G., Harmon, J.B., Stump, J.M., Lynes, T.E., Marvel, J.P. and Smith, D.H. (1969) The toxicology and pharmacologic properties of amantadine hydrochloride. *Tox. appl. Pharm.* **15**, 642-665.

EFFECTS OF TRAINING ON PHARMACOKINETICS OF ANTIPYRINE IN HORSES

T. M. Dyke, R. A. Sams and K. W. Hinchcliff

Analytical Toxicology Laboratory, The Ohio State University, Columbus, Ohio 43210-1089, USA

OBJECTIVES

A model was developed, studying training-induced changes to the pharmacokinetics of antipyrine, to predict potential influences of training on the pharmacokinetics and body fluid concentrations of drugs with low hepatic extraction in horses. The null hypotheses tested were that treadmill training of horses would not alter the plasma clearance or the urinary metabolite excretion of antipyrine (AP). We expected that training would induce hepatic P450 enzymes, leading to changes to antipyrine plasma kinetics as a result of exercise.

MATERIALS AND METHODS

Fourteen adult Standardbreds were split into 2 groups: a 'training' group that underwent treadmill training for 5 weeks, and an 'untrained' group that remained in box stalls for the same period.

Incremental exercise tests were performed prior to drug administration to determine maximal oxygen consumption (VO_2max), speed vs. oxygen consumption, speed producing blood lactate concentrations of 4 mmol/l (VLa_4) and blood lactate concentrations at 8 m/s (La_8). Antipyrine was administered iv (20 mg/kg bwt) to resting horses in both groups before and after the training period. Blood and urine samples were taken for 8 h. Training consisted of treadmill exercise for 18 min/day (2 min at 3 m/s, 10 min at a speed estimated to allow 60% VO_2max , 3 min at speed estimated to allow 90% VO_2max , a further 2 min at 60% VO_2max followed by 1 min at 3 m/s), 6 days/week for 5 weeks. Incremental exercise tests were performed after the training period to determine training-induced changes to maximal oxygen consumption, VLa_4 and La_8 . Plasma samples were analysed for antipyrine concentrations using normal-phase high performance liquid chromatography. Data from plasma antipyrine vs. time data were analysed using weighted, non-linear regression and estimates of pharmacokinetic variables derived. Urine samples were frozen in sodium acetate/sodium metabisulphite. Thawed, extracted aliquots were analysed for conjugated and

unconjugated antipyrine, norantipyrine and 4-hydroxyantipyrine by reverse-phase HPLC. Renal excretion of antipyrine and metabolites over 8 h were calculated.

Maximal oxygen consumptions, VLa_4 , La_8 , plasma antipyrine clearances, volumes of distribution and urinary excretion of substances within and between groups were compared using Wilcoxon signed-rank tests and Mann-Whitney rank-sum tests respectively ($P < 0.05$).

RESULTS

Changes in maximal oxygen consumptions, VLa_4 and plasma clearances were significantly different between groups as a result of training. Antipyrine plasma concentrations were best described by a 2 exponential equation. Volumes of distribution of antipyrine and urinary excretion of antipyrine and metabolites were not significantly different as a result of training.

CONCLUSIONS

Apart from increases in plasma clearance as a result of training and decreases as a result of box rest, we were unable to detect training-induced changes in antipyrine pharmacokinetics. There was considerable intra- and inter-individual variability in excretion patterns of antipyrine and its 2 major metabolites in horses.

IMPLICATIONS

1. The low renal excretion of antipyrine and metabolites in horses may have limited the usefulness of this model in predicting training-induced effects on pharmacokinetics of drugs with poor hepatic extraction.
2. Further studies are to be conducted evaluating drugs of regulatory interest, eg phenylbutazone, to study the potential for training to influence the pharmacokinetics of drugs.

ACKNOWLEDGEMENTS

Supported by the Ohio Thoroughbred Research Fund and the Ohio Standardbred Research Fund.