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Amantadine and equine influenza: pharmacology, pharmacokinetics and neurological effects in the horse

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Summary

Amantadine is an antiviral agent effective against influenza A viruses. We investigated 1) the antiviral efficacy, 2) analytical detection, 3) bioavailability and disposition, 4) pharmacokinetic modelling and 5) adverse reactions of amantadine in the horse.

***In vitro*, amantadine and its derivative rimantadine suppressed the replication of recent isolates of equine-2 influenza virus with effective doses (EDs) of less than 30 ng/ml. Rimantadine was more effective than amantadine against most viral isolates; we suggest a minimum plasma concentration of 300 ng/ml of amantadine for therapeutic efficacy. *In vivo* an i.v. dose of amantadine 15 mg/kg bwt produced mild, transient CNS signs which were no longer apparent after 30 min. Amantadine administered at a dose of 15 mg/kg bwt was established as the maximum safe single i.v. dose. However, if repeated i.v. administration of amantadine is required no more than 10 mg/kg bwt t.i.d. should be used.**

The maximal safe plasma concentration of amantadine was not evaluated but is probably greater than 2000 ng/ml and possibly greater than 4000 ng/ml. On the other hand, horses with lower seizure thresholds, or those on medications that lower seizure thresholds, may be at increased risk of amantadine-induced seizures, which show few premonitory signs and are rapidly fatal.

After i.v. administration of amantadine 10 mg/kg bwt, the disposition kinetics were well fitted by a 2-compartment open model. The estimated peak plasma concentration after this dose was about 4500 ng/ml, the volume of distribution at steady-state (Vdss) was (mean ± s.d.) 4.9 ± 1.9 l/kg bwt and the β phase half-life was 1.83 ± 0.87 h. Computer projections of plasma amantadine concentrations after i.v. administration of amantadine at a dose of 10 mg/kg bwt t.i.d. at 8 h intervals suggest peak plasma concentrations of 4000–5000 ng/ml and troughs of less than 300 ng/ml will be achieved.

Amantadine administered orally at 10 mg/kg bwt and 20 mg/kg bwt showed mean oral bioavailability of about 40–60% and a plasma half life of 3.4 ± 1.4 h; however, there was substantial inter-animal variation in bioavailability. Projections based on the kinetics observed in individual animals suggest that some animals readily maintain effective plasma concentrations of amantadine after oral administration of 20 mg/kg bwt t.i.d. On the other hand, animals in which amantadine is poorly bioavailable may require up to a 6-fold (120 mg/kg bwt) increase in the oral dose to achieve effective blood concentrations. Withholding food for 15 h did not reduce these inter-animal differences in bioavailability.

Our results showed that simple dosing with oral amantadine will not yield effective plasma concentrations in all animals. While i.v. administration yielded more reproducible plasma concentrations, care should be taken to see that the seizure threshold is not exceeded. In acute situations, i.v. administration (5 mg/kg bwt) every 4 h should maintain safe and effective plasma and respiratory tract concentrations of amantadine.

Introduction

Amantadine (1-adamantanamine) is an aliphatic primary amine with a pKa of 10.1 and a molecular weight of 151.26. Originally discovered in the mid 1960s, amantadine was found to have a number of pharmacological properties, including inhibition of influenza viral replication (Aoki and Sitar 1988). The antiviral

TABLE 1: Double blind cross over neurological evaluations after administering amantadine HCL i.v. (15 mg/kg bwt) to 6 horses

Treatment group (Week 1)	Examination status post dose
1	No neurological signs; no change after dosing
2	No neurological signs; no change after dosing
3	Enhanced truncal sway at the walk; interfered behind on the tight circle
*	
Treatment group (Week 2)	
1	Mare slipped on the concrete; pivoting was worse than before dosing
2	Mare stumbled badly; tucked rear quarters on the tight circle pivoted on the rear limbs on the tight circle
3	Short steps behind; no real change after dosing

3 Controls: No change in neurological status in weeks 1 and 2

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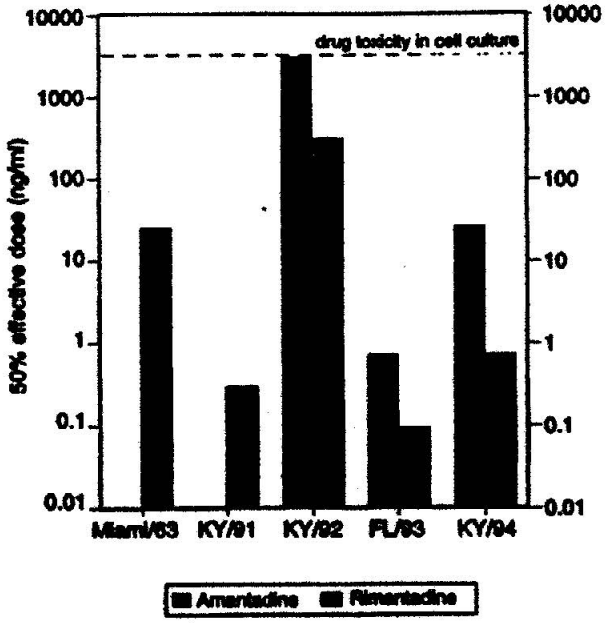

Fig 1: Sensitivity of recent strains of equine-2 influenza viruses to amantadine and rimantadine using culture infectivity reduction (TCID50 reduction) assay as described in methods. The solid bars indicate virus sensitivity to amantadine and the hatched bars represent virus sensitivity to rimantadine.

use of this agent has been implemented in the prophylaxis and treatment of human influenza virus-A2, but has been reported to cause transient neurological effects in man after chronic oral administration (Hayden *et al.* 1981). In human medicine, amantadine is widely used in the prophylaxis of influenza in high risk populations, such as nursing home residents (Dolin *et al.* 1982).

Influenza is a highly significant acute respiratory disease in the horse. Individual cases are generally mild, but individuals may be predisposed to severe secondary bacterial infections. A recent equine influenza outbreak in Hong Kong suspended racing for one month and resulted in estimated losses of $100 million (Chambers *et al.* 1994). Equine influenza can be an important cause of morbidity and mortality in foals and mature horses. In individual animals of value, prophylaxis or treatment with amantadine or rimantadine could be beneficial because vaccination is only partly effective in preventing the disease.

Amantadine and rimantadine are antiviral agents with potential for prophylactic and therapeutic use against susceptible strains of equine influenza. Recent availability of an ELISA test (Directigen Flu A)[1] for 'on the spot' diagnosis of the disease now makes prophylaxis and early treatment of equine influenza possible (Chambers *et al.* 1994). Clinical signs could be ameliorated by early treatment. Affected individuals and animals exposed to challenge might be protected by appropriate doses of these agents and simultaneously vaccinated to stimulate development of natural immunity. These agents also provide protection against all susceptible viral strains, whereas vaccines are generally protective only against virus strains closely related to those included in the vaccine (Nahata and Brady 1986).

The therapeutic range and dosing schedules that yield effective therapeutic plasma concentrations of amantadine must first be established. We, therefore, investigated the analytical detection, bioavailability, pharmacokinetics, and clinical signs following administration of amantadine to horses in order to establish prophylactic and therapeutic protocols.

TABLE 2: Pharmacokinetic parameters of amantadine after administration i.v. (10 mg/kg bwt) and *per os* (20 mg/kg bwt) to Thoroughbred mares (mean ± s.d.)

Dose (mg/kg bwt)		Clearance (ml/min/kg)	$V_{steady\ state}$ (l/kg)	$T_{1/2}$ (β-phase)	Bioavailability
Intravenous	10	36.8 ± 12.4	4.87 ± 1.9	1.83 ± 0.87	
Oral	20	185.2 ± 183.6	-	3.37 ± 1.42	60–40%

Materials and methods

Experimental approach

Five separate experimental approaches were utilised in order to 1) develop a sensitive gas chromatographic method to determine the disposition and pharmacokinetics of amantadine, 2) determine *in vitro* the antiviral efficacy of amantadine and rimantadine against selected equine influenza isolates, 3) establish toxicity thresholds for neurological signs by administering 2 i.v. doses of amantadine (10 and 15 mg/kg bwt), 4) establish the pharmacokinetics of amantadine by administering amantadine i.v. (10 mg/kg bwt) and *per os* (10 and 20 mg/kg bwt) with and without feed withdrawal. Plasma samples were taken at specific times pre- and post medication and were subjected to chromatographic and pharmacokinetic analysis and 5) a computer simulation of plasma concentrations of amantadine likely to be found after repeated dosing with this agent was calculated from pharmacokinetic data derived from i.v. and *per os* data measured in approach 4.

Horses

Six mature Thoroughbred mares weighing between 412–603 kg were used. These animals were in good health and kept at pasture until the morning of the experiment. They were maintained on a regular seasonal anthelmintic programme and subjected to clinical examinations by a veterinarian before and after treatments. Oral administrations were performed via a stomach tube, and i.v. injections administered into the left jugular vein; all blood samples were drawn from the right jugular vein into vacutainer[1] green top (sodium heparin) tubes. The plasma was separated by centrifugation and stored at 4°C until analysed.

Drugs and chemicals

Amantadine HCL was purchased from Aldrich Chemical Company, St. Louis, Missouri. Rimantadine HCL was obtained from Forest Pharmaceutical, St. Louis, Missouri. All chemicals and reagents used in the analysis of plasma samples were HPLC grade or better.

Anti-viral efficacy in vitro

Parallel tests of equine influenza virus isolate sensitivity to amantadine and rimantadine were conducted by tissue culture infectivity reduction ($TCID_{50}$ reduction) assay. The assay medium consisted of Medium 199 supplemented with 0.25% (w/v) bovine serum albumin; 10 mmol/l Hepes buffer, pH 7.3; antibiotics (penicillin/streptomycin/amphotericin B); and TPCK trypsin (1 μg/ml).

In 96-well microtitre plates containing cultured MDCK cells freshly infected with 200 TCID units per well of different equine

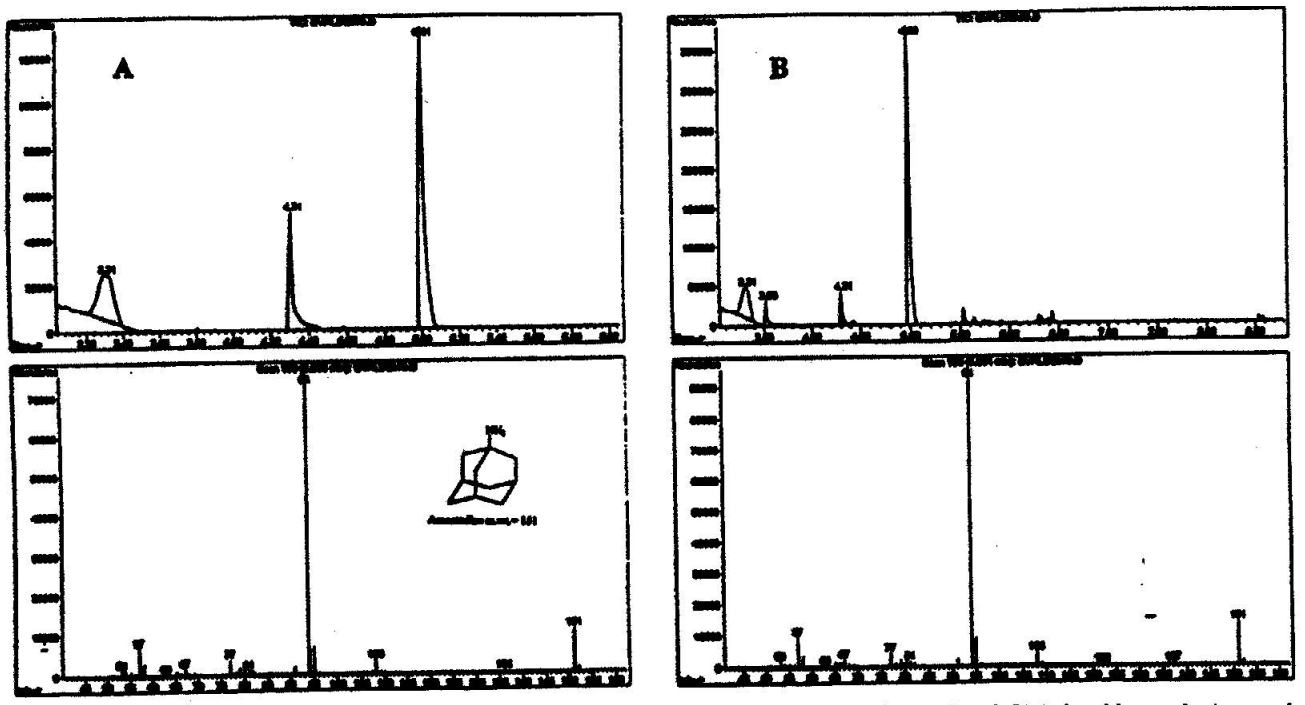

Fig 2: Detection of amantadine by gas chromatography/mass spectrometry. A) Amantadine standard in methanol. B) A dosed horse plasma sample extracted as in methods. For both figures the amantadine retention time is ~5.0 min post injection.

influenza virus strains, half-log serial drug dilutions ranging from 0.1 to 30,000 ng/ml were added to sextuplicate wells, and the cells were incubated in the presence of drug at 37°C. After 72 h, the wells were stained with crystal violet in formalin and the maximum drug dilution producing inhibition of viral cytopathic effect in 50% of replicate wells was calculated.

Miami/63 virus, the prototype equine-2 influenza virus, was a laboratory strain extensively passaged in eggs and, in our hands, adapted to growth in MDCK cells. KY/92, KY/93, and KY/94 viruses were isolated in our laboratory using embryonated eggs from equine influenza outbreaks in Kentucky. Following egg passage No. 2, these viruses were also adapted to growth in MDCK cells. FL/93 virus, from an equine influenza outbreak in Florida, was isolated in our laboratory directly in MDCK cells. For all viruses, stocks from MDCK passage No. 3 or No. 4 were used for drug resistance assays.

Analytical methods

Drug extraction: Each plasma sample (3 ml) was added to a screw-cap glass test tube with the appropriate amantadine standards and internal standard (rimantadine). Ammonium hydroxide 50% (4 ml) was next added to the standard and sample tubes to adjust the pH to 11.0. Dichloromethane solvent (5 ml) was added, and the samples were mixed by rotation for 1 h at room temperature and centrifuged for 40 min at 4°C at 2000 *g*. The supernatant was aspirated off and the organic solvent layer transferred to a glass conical tube. The tubes were placed in a water bath (40°C) and carefully blown to dryness under a steady stream of nitrogen. The sample was then reconstituted in 100 µl methanol.

Gas chromatography: A gas chromatograph (Varian 3400) equipped with a nitrogen-phosphorus detector and autosampler was used to analyse all extracts of the plasma samples for amantadine. The chromatograph contained a 15 m x 0.25 mmol/l i.d. silica capillary column (Rtx-5)[2]. A programmable temperature was used with an initial column temperature of 80° held for 1.0 min. The oven temperature increased 20°C/min until it reached 250°C and was held for 10.5 min. The injection port (splitless) and detector were maintained at 220 and 320°C, respectively. The carrier gas (nitrogen) flow was 30 ml/min, the detector air flow was 175 ml/min and the detector hydrogen flow was 4 ml/min. The peaks were evaluated by the Varian Star chromatography software system.

Mass spectrometry: The verification of amantadine was performed by gas chromatography/mass selective detection (6890 Series 3 MSD)[3]. The chromatograph contained a 30 m x 0.25 mmol/l i.d. siloxane capillary column (HP-5MS 5% phenyl methyl siloxane)[3]. A programmable temperature was used with an initial column temperature of 60°C held for 2.0 min. The oven temperature increased 50°C/min until it reached 250°C and was held for 5.0 min. The injection port (splitless) was maintained at 100°C with a pressure and purge flow of 8.2 psi and 50.0 ml/min respectively. The carrier gas (helium) flow was 1 ml/min.

Pharmacokinetic analysis

Pharmacokinetic analysis was determined by using the model selection criterion of a one or 2-compartment model regression analysis programme which was weighted, RSTRIP[4]. Area under the curve (AUC) was measured by linear trapezoidal approximation with extrapolation to infinity, and the slope of the log of the terminal half-life was determined by the method of least squares regression. The systemic (Cls) and oral clearances (Clo) was calculated as:

$$Cls = \frac{Dose^{i.v.}}{AUC^{i.v.}} \qquad Clo = \frac{F \times Dose^{po}}{AUC^{po}}$$

The volume of distribution at steady state (Vss) was calculated as

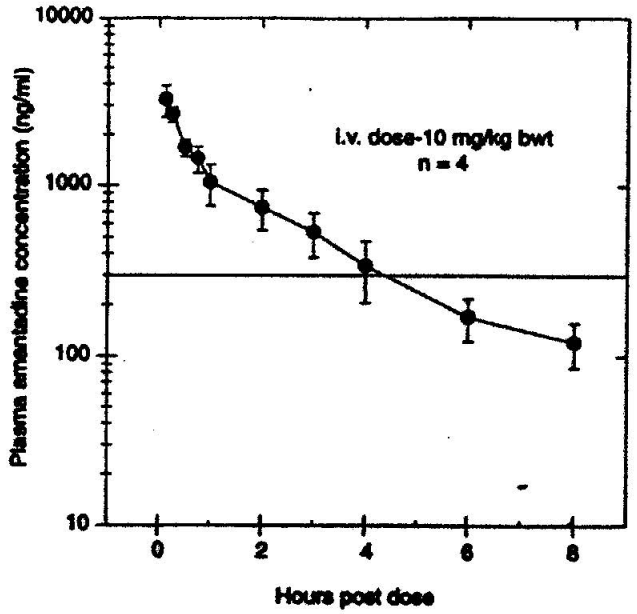

Fig 3: Amantadine was administered i.v. at 10 mg/kg bwt to 4 horses and the plasma concentrations plotted against time. The horizontal solid line indicates the desired plasma level (300 ng/ml) needed for inhibition of viral replication.

$$V_{ss} = \frac{Dose^* AUMC}{(AUC)^2}$$

The bioavailability (F) was calculated as

$$F = \frac{AUC^{po}}{AUC_{i.v.}} * \frac{Dose^{i.v.}}{Dose_{po}}$$

To project the effect of multiple dosing upon peak, trough and mean steady-state concentrations, multiple dose predictions were calculated for animals exhibiting median high and low absorption kinetics of amantadine after oral and i.v. administration using the programme Scientist[4].

Neurotoxicity of amantadine

Determination of the neurological effects of administering high doses of amantadine (15 mg/kg bwt) was performed on 6 horses in a double crossover study. All neurological examinations were carried out by a licensed equine veterinarian who is board certified in internal medicine (Clara Fenger). In this experiment, 3 horses received amantadine 15 mg/kg bwt i.v. and 3 were given a saline placebo injection. The examination, limited to a clinical evaluation, consisted of a cranial nerve examination, muscle symmetry evaluation, placing reaction, gaiting on the straightway with the head raised, circling in tight circles in both directions and finally, backing. Each examination took place before the agent was administered, immediately post i.v. administration, at 30 min and 1 h post injection. One week later the amantadine-treated and control horses were crossed over, and the analysis was repeated. Preliminary toxicity evaluations had been performed after administering amantadine i.v. at 2 dose rates, 10 mg/kg and 20 mg/kg bwt.

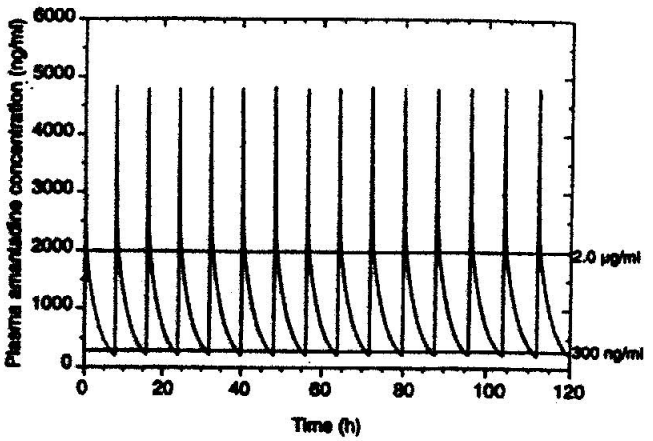

Fig 4: The simulation of multiple i.v. doses of amantadine(10 mg/kg bwt) given to horses every 8 h based on calculated kinetic data. The upper horizontal solid line indicates the beginning of the established toxic level for amantadine, while the lower horizontal solid line indicates the minimum therapeutic effective concentration of 300 ng/ml.

Results

In vitro *antiviral efficacy*

Sensitivity of equine influenza viruses to amantadine was first demonstrated by Bryans *et al.* (1966). To determine whether or not contemporary strains of equine influenza viruses have retained sensitivity, a number of recent isolates were tested. The data in Figure 1 shows that, with the exception of KY/92 strain, all isolates were inhibited by concentrations of amantadine or rimantadine of 30 ng/ml or less; however, one isolate (KY/92) appeared resistant to concentrations of amantadine and rimantadine up to 300 ng/ml. The antiviral efficacy of rimantadine was greater than that of amantadine. Using a different assay, plaque reduction, KY/92 viral plaquing efficiency was reduced about 75% by 100 ng/ml of amantadine. The results demonstrate that contemporary strains of equine influenza, while variable, are sensitive to inhibition by amantadine and rimantadine. Influenza viruses of equine-2 (H3N8) subtype are the only subtype currently in circulation and are sensitive to amantadine (this work and Bryans *et al.* 1966).

Neurotoxicological evaluation

In horses, preliminary experiments showed that amantadine administered i.v. at a dose of 10 mg/kg bwt produced no obvious adverse effects, while doses of 20 mg/kg bwt or more produced variable CNS effects including seizures. We, therefore, performed a double blind crossover study to evaluate the neurotoxicity of amantadine administered i.v. at a dose of 15 mg/kg bwt. In this experiment, we observed mild and transient CNS signs in 3 of 6 treated horses immediately after i.v. administration of amantadine. However, the signs were no longer apparent at 30 and 60 min after administration (Table 1). Cranial nerve abnormalities were not noticed in any of these horses. Signs of CNS effects included stumbling, inconsistent limb placement, dragging of hind toes and weakness in the lower back muscles. It was concluded that single i.v. administrations of amantadine at a dose of 15 mg/kg bwt were unlikely to be associated with significant adverse responses in horses.

In preliminary experiments to establish the safe range of amantadine administration, one horse had seizures and died

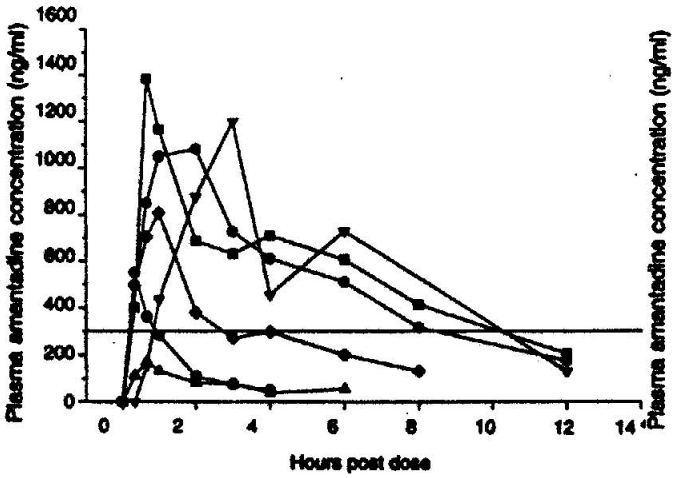

Fig 5: Plasma amantadine concentrations after administering amantadine per os at a 20 mg/kg bwt to 6 horses with feed. The horizontal, solid line indicates the minimum therapeutic effective concentration of amantadine in plasma (300 ng/ml).

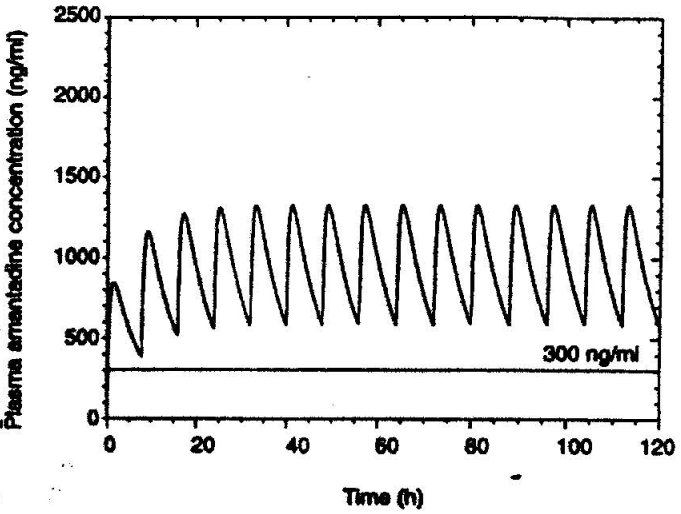

Fig 6: The simulation of multiple oral doses of amantadine (20 mg/kg bwt) given to a horse which exhibited high absorption kinetics. (No. 537) The horizontal, solid line indicates the minimum therapeutic effective concentration of amantadine in plasma (300 ng/ml).

shortly after slow i.v. administration of twice the recommended oral dose (20 mg/kg bwt). In another unrelated experiment, a pony died after eight i.v. doses of 15 mg/kg bwt of this agent at 8 h intervals, despite suggestion that this was a well tolerated dose. The pony had been treated with xylazine prior to administration of amantadine which may have reduced the seizure threshold of this animal.

Analytical methodology

We developed a sensitive and specific method of detecting amantadine in equine plasma using gas chromatography and mass spectrometry (GC/MSD) (Fig 2). The limit of sensitivity of this method was 50 ppb, and the detector response was linear up to 1000 ng/ml. The correlation coefficient for the standard curve was $r = 0.9961$. Because the minimal inhibitory concentration (plasma) of amantadine for antiviral effect is reportedly about 300 ng/ml, this method has more than sufficient sensitivity for the current research project. Recovery of amantadine from plasma samples was about 55%, and the range for the coefficient of variation for this method was between 7–10% after extraction from equine plasma.

To confirm the validity of the nitrogen-phosphorus detection we performed GC/MSD on pure standards of amantadine and on equine plasma samples, obtained from horses dosed with amantadine, following drug extraction as described in materials and methods. The mass spectrum were similar in both cases and matched those seen by Biandrate *et al.* (1972) who detected amantadine in human plasma by GC.

Pharmacokinetic data

After i.v. administration of amantadine at a dose of 10 mg/kg bwt, plasma concentrations were well fitted by a 2-compartment open model. Plasma concentrations of amantadine peaked at about 3.2 µg/ml 5 min after injection (Fig 3) and then declined in a bi-exponential fashion. Plasma concentrations of amantadine were followed for about 8 h post injection, after which time they fell below the limit of detection (50 ng/ml) of the method. Data analysis showed that the systemic clearance, volume of distribution and terminal elimination half-life were (mean ± s.d.) 36.8 ± 12.4 ml/min/kg bwt, 4.9 ± 1.9 l/kg bwt and 1.83 ± 0.87 h, respectively (Table 2).

A simulation of the predicted plasma concentrations of amantadine after repeated i.v. administration of amantadine at 10 mg/kg bwt is presented in Figure 4. Trough concentrations of amantadine fell below the suggested 300 ng/ml minimum at 4–5 h after amantadine administration, leaving a significant period during which viral replication might not be inhibited. More troubling, however, was that the peak plasma concentrations predicted by this model, 4500 ng/ml, were high for an agent with neurotoxic potential. Furthermore, increasing the dose of amantadine to 15 mg/kg bwt q. 8 h to prevent the trough concentration from falling below 300 ng/ml would increase the peak plasma concentrations to about 6700 ng/ml.

We next evaluated the bioavailability of amantadine after administration at a dose of 10 mg/kg bwt via a stomach tube. Analysis of these data (not presented) suggested that the oral bioavailability of amantadine was low, apparently in the order of about 40%. Therefore, we repeated the oral bioavailability experiment using a 20 mg/kg bwt oral dose. In these experiments the horses were allowed free access to hay and water (Fig 5). After this dose, plasma concentrations of amantadine increased rapidly, peaking between 1 and 2 h after administration. Three of the horses showed peak plasma amantadine concentrations reaching 1.2 µg/ml, and therapeutic plasma concentrations were maintained in these animals for 8 h or more. On the other hand, 3 horses had more variable peak plasma amantadine concentrations, although the time to reach peak concentration was comparable in all 6 animals. Therapeutically effective plasma concentrations were not attained in 2 of these horses and only briefly attained in a third. The calculated β-phase half-life after oral administration of amantadine (20 mg/kg bwt) was 3.37 ± 1.42 h as shown in Table 2.

One reason for the very large variation of the bioavailability of amantadine between horses after administration *per os* is that the presence of food in the intestinal tract may have affected the absorption of this agent. We, therefore, repeated the experiment depicted by Figure 5 with feed and bedding withdrawn 15 h prior to dosing. Analysis of the data (Fig 7) revealed that the plasma concentrations of amantadine peaked later, between 2 and 4 h post dose, but the withholding of feed did not affect the overall bioavailability of amantadine; bioavailability being between

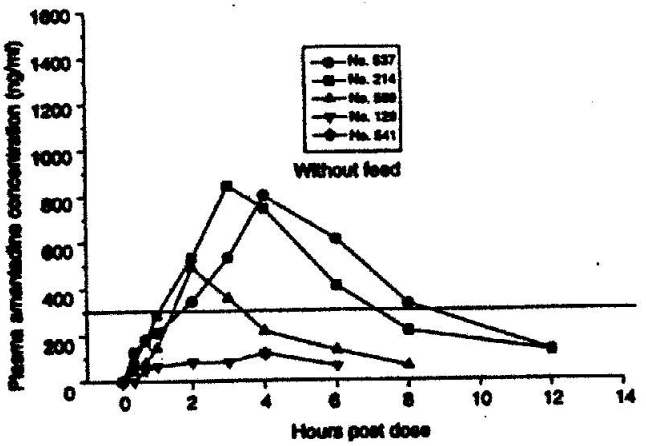

Fig 7: Plasma amantadine concentrations after administering amantadine per os at a dose of 20 mg/kg bwt to 5 horses without feed. The horizontal solid line indicates the minimum therapeutic effective concentration in plasma (300 ng/ml). Horse No. 541 had no detectable amantadine plasma concentrations and a graph was therefore not plotted.

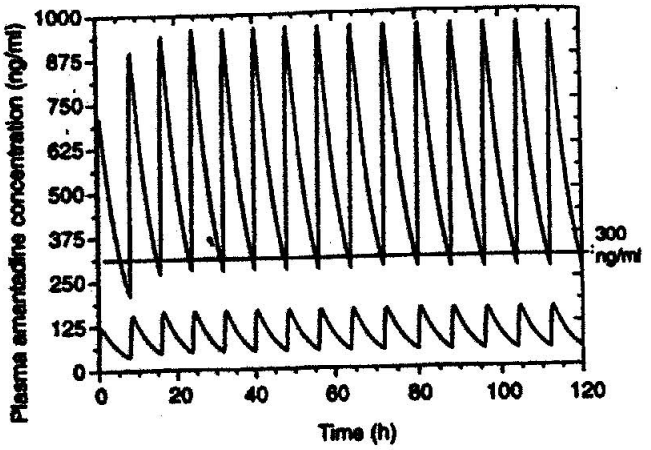

Fig 8: The simulation of multiple oral doses of amantadine (20 mg/kg bwt - lower set of curves; 120 mg/kg bwt - upper set of curves) given to a horse which does not readily absorb amantadine (No. 541). The horizontal, solid line indicates the minimum therapeutic effective concentration of amantadine in plasma (300 ng/ml).

40–60% with feed and 39% without feed.

Discussion

The present study was designed to establish a pharmacokinetic basis for amantadine as a prophylactic and therapeutic agent in the treatment of equine influenza. We needed to establish 1) a sensitive analytical method, 2) bioavailability, pharmacokinetics and disposition in the horse, 3) plasma concentrations associated with significant adverse reactions and 4) dosage schedules that maintain effective antiviral concentrations in equine plasma and respiratory fluids. The ultimate goal of these experiments was to develop prophylactic or treatment dosing schedules which can be used by veterinarians to treat effectively an outbreak of equine influenza.

Central nervous system-based adverse reactions to amantadine are well known in human medicine and, similarly, we observed that high concentrations of amantadine produce acute seizures and even death in horses. Our work showed that amantadine given i.v. 10 mg/kg bwt produced no obvious adverse responses, while doses of >15 mg/kg bwt produced variable CNS effects including unpredictable seizures. In a double blind crossover study involving a single dose of amantadine (15 mg/kg bwt) given i.v. we observed only mild and transient signs of CNS distress; however, we strongly suggest that a dose not more than 10 mg/kg bwt be administered i.v. and that the dose be administered in 2 phases with at least 15 min between injections.

We did not study the specific plasma concentration at which amantadine precipitates seizures in horses, but this concentration is clearly greater than 2000 ng/ml and appears to be greater than 4000 ng/ml. In this regard, the human literature (Bleidner *et al.* 1965) suggests that important factors such as individual variability in patient seizure thresholds or the medication status of each patient should be carefully considered before a proper dose is chosen to avoid complications. For example, some medications, such as the phenothiazine tranquilizers, lower seizure thresholds.

The apparent volume of distribution (4.9 l/kg) (Tobin 1981) of amantadine following its administration suggests this agent is distributed into a volume about 8 times greater than that of body water. Inspection of the disposition curve after i.v. administration at this dose shows that plasma amantadine concentrations fall below 300 ng/ml after about 4 h. Based on the *in vitro* antiviral effective doses (Fig 1) and data reported in the human literature (Vernier *et al.* 1969) on amantadine, we selected 300 ng/ml as the minimum therapeutic concentration for this agent in equine plasma and alveolar fluids. Computerised simulations of the plasma concentrations of this agent likely to be found after repeated dosing at 8 h intervals show that the trough concentrations drop below the 300 ng/ml concentration for relatively short periods. On this basis, review of the data of Figure 3 and the projections of Figure 4 suggest that i.v. administration of amantadine at 10 mg/kg bwt q. 8 h is advisable. More conservatively, in an intensive care situation, the dose could be reduced to 5 mg/kg bwt and frequency increased to q. 4 h. This approach would avoid the high plasma concentrations associated with the 10 mg/kg dose. Similarly, i.v. infusion at the same rate should give rise to effective plasma concentrations for acute therapeutic situations.

The information obtained from oral administration of amantadine suggests substantial inter-animal variation in the oral bioavailability of amantadine ranging from very low (approximately 10%) to a maximum of about 70%, which could lead to problems in developing oral dosing schedules for large numbers of animals. Pharmacokinetic projections of these data show that horse No. 537 rapidly attained and maintained effective plasma concentrations of amantadine after oral administration of this agent (Fig 6). Conversely, horse No. 541 will never attain effective plasma concentrations unless the dose of amantadine is substantially increased. In fact, computer modelling suggests that a horse such as horse No. 541 would need to be dosed with 6-fold more amantadine to achieve trough plasma concentrations of amantadine greater than 300 ng/ml (Fig 8).

Overall, these data show that for effective therapeutic use, a strategy must be devised to maintain the plasma concentrations of amantadine above 300 ng/ml and below 1500 ng/ml. If plasma concentrations fall below the MIC, the drug is ineffective; on the other hand, if the plasma concentrations rise too high, the animal is at risk of seizures. One approach to this problem would be to develop a simple ELISA test for amantadine that would allow for direct monitoring of plasma concentrations of this agent. A second approach, which we are currently investigating, would be to

nebulise amantadine (or rimantadine) directly into the respiratory tract, the site of influenza virus infection, to obtain high, effective local concentrations in the respiratory tract and avoid developing high systemic blood concentrations.

Drug resistance to antiviral medications is always a concern when prophylactic treatment is used in the field (Klimov *et al.* 1991). In the case of influenza, mutations may occur in the M2 protein, the site of amantadine-rimantadine activity. Hay *et al.* (1985) have shown that resistance is determined by a single amino acid substitution at one of 4 positions in the transmembrane domain of the M2 protein. Hayden *et al.* (1989) has also reported on the development of resistant strains of human influenza after periods of rimantadine treatment in families. As such, resistance may develop and become relevant in the future if these medications come into wide use in horses.

In summary, review of these data demonstrates that amantadine is effective *in vitro* in inhibiting replication of wild strains of equine influenza virus. *In vivo*, however, there appears to be a relatively narrow range of concentrations between which plasma amantadine must be held to ensure therapeutic efficacy. Plasma concentrations should not drop much below 300 ng/ml to maintain MICs of this agent. On the other hand, plasma concentrations should not rise above 2000 ng/ml to avoid seizures precipitated by high concentrations of amantadine. Oral administration has the potential to be effective in animals that absorb amantadine well from the gastrointestinal tract; on the other hand, animals that show poor oral bioavailability of amantadine need much larger doses of amantadine to maintain effective blood concentrations of this agent. Rimantadine appears to be more effective than amantadine *in vitro* and is likely to be the superior prophylactic and therapeutic agent as long as its bioavailability, disposition and adverse reaction characteristics are not significantly worse than those of amantadine. We are currently evaluating these characteristics of rimantadine in the horse.

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Manufacturers' addresses

[1]Becton-Dickinson, Rutherford, New Jersey, USA.
[2]Resnik Corp, Bellefonte, Pennsylvania, USA.
[3]Hewlett Packard, Palo Alto, California, USA.
[4]Micromath, Salt Lake City, Utah, USA.

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Amantadine in man and horse - can we learn from each other?

Perhaps a positive side effect of the current political controversy over the veterinary and, possibly, medical crisis confronting our society in the potential link between bovine spongiform encephalopathy (BSE) and Creutzfeldt Jakob disease (CJD), may be a more co-ordinated and discussive approach between veterinary and medical virologists.

Veterinarians and human virologists have much in common with respect to discussions of influenza. Not least was the observation by a veterinarian of the movement of pandemic influenza A virus from man to pigs in 1918–1919; and the concern ever since that the same virus (which still infects pigs) may move back to man. Again many of us, although not the present writer, believe ardently that the new pandemic influenza A virus may emerge from the virological mixing bowl of pigs co-infected with avian, pig or indeed equine influenza A viruses. Indubitably, the isolation in the laboratory of the first influenza A virus in London in 1934 depended completely on the ferret (Stuart-Harris *et al.* 1985).

In this vein, the paper in the current issue (p 104) by Rees *et al.* (1997) raises issues commonly discussed amongst virologists interested in human infections with influenza A, concerning prevention with antiviral drugs. What are optimal dosing schedules with amantadine, how effective is chemoprophylaxis likely to be against a hypervariable virus such as influenza A, and what are the possible side effects? Veterinary and medical virologists are certainly united in their appreciation of the dire effects of influenza A virus in the communities under their care as regards morbidity, mortality and economics. In both communities, inactivated influenza vaccine forms the main method of prevention but their effectiveness is certainly less than 100%.

The requirement for rapid diagnosis is yet another common theme in human and veterinary respiratory virology. The new influenza A and B ELISA and PCR tests will help tremendously in what has been, up to now, a diagnostic nightmare. Sir Charles Stuart-Harris, the Sheffield respiratory physician and virologist, and part of the London team that first isolated influenza A in 1934, was one of the first to try and dissect out the respiratory syndromes and the plethora of viruses causing them (Stuart-Harris *et al.* 1985).

Amantadine (Symmetrel)[1] has been licensed as an antiviral agent for human use in Europe and the USA for nearly 3 decades, whereas the structurally related molecule rimantadine, although widely utilised in the former USSR, has had less penetration, at least to date in Europe, the USA and the UK. This situation may change now because of its registration in the USA and France. Most of the extensive clinical and pharmacological data of these 2 drugs dates back several decades (Oxford and Galbraith 1983). What is entirely new is the establishment of their antiviral target protein which turns out, rather unexpectedly, to be the influenza M2, a curious protein which is present in the lipid of the virus and in intracellular vesicles in infected cells and may play a role as an ion channel. Amantadine could act by blocking this ion channel, thereby preventing natural acidification of the input virus which, in turn, normally allows the dissociation of the internally situated virus M, NP and RNA complex. In the presence of amantadine, infection of the cell is aborted (Hay 1992; Pinto *et al.* 1992). Can the human virologist, therefore learn anything from the horse and vice versa? Is there much to learn? In answer to the first question, there probably is, but more data are required for a definitive opinion; and in answer to the second question, there is much to be learnt.

Obviously we may not be able to move data directly from human to horse, or vice versa, given the vastly different digestive systems and the exclusive use of oral administration of amantadine or rimantadine in man. But where some light can be thrown is on the subject of serious side effects and of precise dosing schedules in individuals, be they horse or man. There is no more serious side effect than death; and in the current paper by Rees *et al.* (1997) overdosing of a horse with amantadine resulted in such an end point. This is worrying to a human virologist, but not totally unexpected and, quite frankly, we need to know why and at what drug concentration this occurred. We also need to be totally clear about the most serious but fortunately extremely rare side effect noted so far in man, namely seizures. From the beginning the drug was known to have mild neurological side effects, not so noticeable as to become a 'pleasure' drug, but worrying enough. But it needs to be appreciated that, as with vaccines, antiviral agents against influenza are mainly advised in 'at risk' groups. There constitute persons with a range of chronic metabolic and medical disorders, such as malfunctioning kidney or heart, as well as diabetes, asthma or bronchitis, which put them at serious risk of incapacitating disease or

death. Age itself is not considered to place a person at risk, although it has to be acknowledged that death rates from influenza pneumonia rise steeply from age 75 years and over. Twenty-five thousand people died of influenza in the 1989–90 outbreak in the UK alone. The intention in man is, therefore, to utilise anti-influenza antiviral agents to supplement vaccine in order to prevent death or serious complications. Against such a stern test, the drug level must be as high as possible to maintain a confident antiviral concentration in respiratory tissues. On balance, a few side effects could be tolerated. This brings us to the important issue of balancing potential risks versus benefits.

Extremely rarely, amantadine and rimantadine can both cause and precipitate seizures in individuals prone to them and this has always been a consideration for clinicians while balancing risk versus benefit in an elderly person. It has also led to an appreciation that being dosage dependent, amantadine and rimantadine dosage must be individualised for elderly patients and, particularly, for those with chronic renal malfunction. One of the earlier reports noted an increased seizure frequency in children on anticonvulsants who had serum levels of 2000 ng/ml of amantadine. There is a marked difference in pharmacology between rimantadine and amantadine which is often forgotten, namely that in man more than 90% of amantadine is excreted unchanged, whereas approximately 75% of rimantadine is metabolised by the liver. Both drugs and their metabolites are excreted by the kidney. Amantadine is excreted unchanged in the urine by glomerular filtration and tubular secretion. Renal clearance is, therefore, reduced substantially in individuals with renal insufficiency and a reduction in dosage is recommended for patients with creatine clearance less than 50 ml/min. In addition it is recommended that such persons should be observed carefully in order that adverse reactions can be recognised promptly and either the dose further reduced or the drug discontinued. In general, amantadine dosage is 5 mg/kg bwt/day *per os* b.i.d. for children age 1–9 years, 100 mg b.i.d. for children age 10–13 years, 100 mg b.i.d. for adults and >100 mg per day for those over age 65 years. Dosage recommendations for rimantadine are very similar. It took a long time to dawn on us that most studies with these 2 drugs had been carried out in strappingly healthy college students, many of them of large size compared, for example, to elderly frail women in the 'at risk' group.

The description of seizures in horses administered 20 mg/kg bwt amantadine i.v. is worthy, therefore, of note and clear thinking. Very relevant is the plasma level causing seizures in horses which approximates to, or is greater than, 4000 ng/ml. This high level, compared to the human dose of 200 mg and corresponding plasma level of 500 ng/ml relates to i.v. usage in the horse. Peak plasma concentrations of 200–300 ng/ml occur in man after a single 100 mg dose and increase to an average of 500–700 ng/ml at a steady state on 100 mg b.i.d. *per os* amantadine dosage in human subjects with steady state trough plasma concentrations averaging 300 ng/ml. In elderly subjects the peak plasma concentration is on average 50% higher, which is why dosage reduction to 100 mg or less daily is recommended for this group.

Most equine influenza A viruses are inhibited *in vitro* by 30 ng/ml or less amantadine. Many human influenza A viruses are inhibited by 10 ng/ml and may, therefore, be more sensitive to inhibition. In fact this aspect is worthy of further side by side comparison, because modification in the assay technique can easily result in 10-fold variation of sensitivity. The authors of the current study, reported in this issue, utilised a sensitive and specific method of detecting the drug by chromatography and GC/MSD. They found that plasma concentrations of drug peaked at 3200 ng/ml, 5 min after i.v. injection and then declined in a big exponential fashion. The main problem was to eliminate dramatic drug troughs without creating potentially dangerously high peaks of drug. Oral administration allowed therapeutic plasma concentrations to be maintained for longer periods without the dramatic highs but, unfortunately, there was marked variation between horses.

One very positive virtue of this class of drugs in man and the mouse is their preferential concentration in fluid in the upper respiratory tract following oral dosing. Here levels in the lung may exceed those in the plasma by 15-fold. An analysis by Rees *et al.* (1997) of their own data and computer based projection, would be i.v. administration of 10 mg/kg bwt amantadine every 8 h for prophylaxis in the horse. This would be approximately 7 times higher than a human dose which is, in contrast, given *per os*. It seems a high level of drug in the horse and a suggestion for future work, therefore, straight from the horse's mouth, would be to study carefully amantadine levels in respiratory secretion as well as plasma, to repeat the work with rimantadine and retest 'street' equine influenza A viruses for sensitivity to the drug. We can then continue a useful dialogue between human and horse virologists.

Manufacturer's address

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Further reading

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