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Pharmacokinetics and Bioavailability of Amantadine in the Horse: A Preliminary Report

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Though human preparation is quite expensive ($600/day per horse), amantadine is an equine aeromask candidate. It prevents viral replication but has poor bioavailability (39%) and can be toxic to the central nervous system when given systemically. Authors' address: Maxwell H. Gluck Equine Research Center, Dept. of Veterinary Science and the School of Pharmacy, University of Kentucky, Lexington, KY 40506.

1. Introduction

Equine influenza is a significant acute respiratory disease in horses. Although individual cases are generally mild, they can predispose horses to severe secondary bacterial infection. A recent equine influenza outbreak in Hong Kong suspended racing for 1 month and resulted in estimated losses of $1 billion. In individual herds, equine influenza is also an important cause of morbidity and mortality in foals and adult horses. Vaccination is only partially effective in preventing the disease.

Amantadine and rimantadine are antiviral agents with specific prophylactic and therapeutic actions against susceptible strains of equine influenza.[1] Acute outbreaks can be prevented with these agents, and the clinical disease can be ameliorated by early treatment. The recent availability of the B-D Directigen FluA enzyme-linked immunosorbent assay (ELISA) test for equine influenza makes prophylaxis and early treatment of equine influenza possible.[2]

We investigated the bioavailability and pharmacokinetics of amantadine in horses to develop prophylactic and treatment protocols for these agents. In this scenario, influenza is diagnosed by B-D Directigen FluA ELISA, the affected and exposed animals are protected by appropriate doses of these agents, and they are vaccinated to stimulate development of natural immunity. Such a protocol should substantially reduce the morbidity, mortality, and economic loss from this disease.

2. Materials and Methods

Six mature Thoroughbred mares weighing 413–602 kg were used for this study. All horses were acclimated to their stalls 24 h prior to experimentation. Amantadine HCL[a] was prepared in filtered water and sterilized prior to intravenous (10 and 15 mg/kg) and oral administration (20 mg/kg). Rimantadine HCL[b] was used as the internal standard during analysis.

A. Analytical Method

Blood samples were collected in sodium heparinized tubes following intravenous and oral dosing at 0, 7.5, 15, 30, 45, and 60 min and at 1, 2, 3, 4, 6, 8, and 24 h following administration. Plasma samples underwent liquid–liquid extraction; the extract was evapo-

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rated to dryness under nitrogen and reconstituted in methanol; analysis was performed by gas chromatography with nitrogen-phosphorus detection. The limit of detection was 50 ng/ml, and the standard curve was linear over 1 log unit.

B. Viral Sensitivity

Viral plaque assays were performed with Madin–Darby canine kidney (MDCK) cells, and several isolated field strains of equine influenza viruses. Serial dilutions of amantadine and rimantadine were added to microtiter wells containing MDCK cells infected with virus and were incubated for 3 days. Minimum inhibitory concentrations of drugs were shown by the presence or absence of a viral cytopathic effect.

C. Pharmacokinetic Analysis

Pharmacokinetic analysis was performed by the fitting of sets of data points from individual animals to either one or two compartment open models, using a Micromath BSTRIP pharmacokinetic modeling program. All rate processes were assumed to be first order, and all reported pharmacokinetic parameters are based on our preliminary analysis.

3. Results and Discussion

We developed a sensitive and specific analytical method for amantadine and rimantadine in equine plasma that detected these agents at low concentrations (50 ng/ml). Because the minimum effective plasma concentration of amantadine that inhibits influenza viral replication is 300 ng/ml in humans,[3] this method is sufficiently sensitive to detect this drug in equine plasma.

We tested the antiviral efficacy of amantadine and rimantadine against field equine influenza isolates. Most isolates were sensitive to amantadine; all isolates were up to ten times more sensitive to rimantadine.

Amantadine and rimantadine cause central nervous system (CNS) side effects in humans.[4] In horses, preliminary research showed that a dose of 10 mg/kg iv produced no obvious adverse responses, whereas doses of 20 mg/kg or more produced variable CNS effects. In a double-blind, cross-over study, we evaluated the neurotoxicity of amantadine 15 mg/kg iv. In three of six horses, mild and transient CNS symptoms occurred that were no longer apparent at 30 and 60 min after bolus intravenous administration. This suggests that doses up to 15 mg/kg of amantadine are safe for slow intravenous administration. However, because of the narrow therapeutic range of this agent, we suggest in a clinical situation that the intravenous dose should be split and administered in two phases with 5–10 min between injections.

We described the pharmacokinetics of this agent after rapid intravenous administration of 10 mg/kg. Plasma concentrations peaked at 4500 ng/ml and declined in a biexponential fashion, with a volume of distribution of 4.9 L/kg. Plasma concentrations of amantadine remained above the critical 300 ng/ml plasma concentration until 4.0 h after dosing, suggesting that intravenous administration of amantadine at 15 mg/kg of 8 h should provide effective prophylactic and therapeutic plasma concentrations of amantadine.

Oral administration of 20 mg/kg of amantadine yielded effective plasma concentrations of amantadine for 8 h after dosing in three of six horses, but less useful plasma concentrations in the remaining horses. Mean oral bioavailability of amantadine was 39%.

Clinically these data suggest that, on an interim basis, the intravenous administration of 10 mg/kg of amantadine three times a day with each administration split into two doses should produce effective concentrations of this agent in horses for the period required for treatment. Although oral administration of 20 mg/kg of amantadine produced effective and prolonged plasma concentrations of amantadine in three horses, plasma concentrations were subtherapeutic for significant periods in the other three horses.

The following is an example of how a clinician may approach a suspected outbreak of equine influenza. The clinician could be called to a farm that has two yearlings in a barn that have been coughing and have an acute fever of 103 °F. With equine influenza suspected as the problem, the veterinarian would nasal swab the yearlings and perform a Directigen FluA test to determine if influenza virus is present. If the result is positive for influenza, the next question the clinician must ask concerns what to do with the other animals in the contaminated barn once the infected animals have been isolated. Vaccination requires a few weeks before being protective to the animal; therefore, antiviral treatment with amantadine is an option until vaccination is complete.

Currently the amantadine preparations available from DuPont are human preparations consisting of a pill form (100 and 200 mg/pill) and an oral syrup (50 mg/5 ml). The cost to treat one mature Thoroughbred horse with the oral syrup at 20 mg/kg is approximately $600 per day.

This research was supported by grants entitled "Amantadine and rimantadine prophylaxis of equine influenza" and "Hemagglutinin antigen of equine-2 influenza virus" from the Grayson-Jockey Club Research Foundation.

References and Footnotes

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[a]Aldrich Chemical Company, Inc., Milwaukee, WI 53233.
[b]Forest Pharmaceutical, St. Louis, MO 63106.