

Pharmacokinetics of imipramine in narcoleptic horses

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Objective—To validate use of high-performance liquid chromatography (HPLC) in determining imipramine concentrations in equine serum and to determine pharmacokinetics of imipramine in narcoleptic horses.

Animals—5 horses with adult-onset narcolepsy.

Procedure—Blood samples were collected before (time 0) and 3, 5, 10, 15, 20, 30, and 45 minutes and 1, 2, 3, 4, 6, 8, 12, and 24 hours after IV administration of imipramine hydrochloride (2 or 4 mg/kg of body weight). Serum was analyzed, using HPLC, to determine imipramine concentration. The serum concentration-versus-time curve for each horse was analyzed separately to estimate pharmacokinetic values.

Results—Adverse effects (muscle fasciculations, tachycardia, hyperresponsiveness to sound, and hemolysis) were detected in most horses when serum imipramine concentrations were high, and these effects were most severe in horses receiving 4 mg of imipramine/kg. Residual adverse effects were not apparent. Value (mean \pm SD) for area under the curve was 3.9 ± 0.7 h \times μ g/ml, whereas volume of distribution was 584 ± 161.7 ml/kg, total body clearance was 522 ± 102 ml/kg/h, and mean residence time was 1.8 ± 0.6 hours. One horse had signs of narcolepsy 6 and 12 hours after imipramine administration; corresponding serum imipramine concentrations were less than the therapeutic range.

Conclusions and Clinical Relevance—Potentially serious adverse effects may be seen in horses administered doses of imipramine that exceed a dosage of 2 mg/kg. Total body clearance of imipramine in horses is slower than that in humans; thus, the interval between subsequent doses should be longer in horses. (*Am J Vet Res* 2001;62:783-786)

Imipramine is a dibenzazepine compound that is commonly referred to as a tricyclic antidepressant because of its chemical structure.¹ The major pharmacologic action of imipramine is to potentiate the actions of biogenic amines such as norepinephrine, dopamine, and serotonin in the CNS by blocking their reuptake at nerve terminals.¹ Consequently, availability of those biogenic amines increases at nerve terminals, resulting in greater postsynaptic excitation. Imipra-

mine also acts as an antagonist at muscarinic, α_1 -adrenergic, and H₁- and H₂-histaminergic receptors. Collectively, these pharmacologic actions presumably are responsible for the therapeutic effects as well as the adverse effects of imipramine in humans. Imipramine has a narrow therapeutic index, with the recommended therapeutic concentration for humans ranging between 50 and 300 ng/ml.¹

Imipramine has been used to treat horses with narcolepsy and other disorders.²⁻⁴ Narcolepsy, which is primarily characterized by signs of excessive sleepiness and cataplexy, is believed to be the result of alterations in the balance of neurotransmitters that regulate sleep. Sleep regulation is controlled at the level of the brainstem by complex networks that use acetylcholine and biogenic amines (eg, norepinephrine, dopamine, and serotonin).⁵ In general, drugs that stimulate monoamine systems in the brain are effective suppressors of narcolepsy, whereas drugs that stimulate cholinergic activity in the brain exacerbate narcolepsy.⁶ In humans with narcolepsy, excessive daytime sleepiness generally is treated with stimulants such as amphetamines, whereas cataplexy often is treated with tricyclic antidepressants such as imipramine. Imipramine simultaneously inhibits cholinergic activity and stimulates aminergic activity, making it one of the most effective drugs used to control cataplexy in humans.^{1,6}

Despite the fact that imipramine has been used to treat narcolepsy in horses, we are not aware of any reports describing its pharmacokinetic properties in this species. To the authors' knowledge, validated methods for determination of imipramine in equine serum have not been reported. Therefore, the objective of the study reported here was to determine the pharmacokinetic disposition of imipramine in narcoleptic horses after IV administration of a single dose.

Materials and Methods

Animals—Five adult horses that had been donated for use in research at our veterinary teaching hospital and that were members of a research colony were used in the study. The horses (4 geldings and 1 mare) comprised 2 Thoroughbreds, 1 Quarter Horse, and 2 Hanoverians. Horses ranged from 8 to 26 years old (median, 14 years) and weighed between 500 and 620 kg (median, 523 kg). Adult-onset narcolepsy had been diagnosed in each horse, as previously described.⁷ Results of a CBC and serum biochemical analyses for each horse were within reference ranges.

Horses were housed separately in stalls beginning 48 hours prior to the start of the study and continuing throughout the duration of the study. Horses were fed grass hay ad libitum throughout the study and had access to water at all times. The study and all aspects of animal care were approved and monitored by an institutional animal care and use committee.

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Procedure and collection of samples—An initial dose of imipramine hydrochloride^a was administered simultaneously to 2 horses (4 mg/kg of body weight, IV). The dose was calculated by using the following formula:

$$\text{Dose} = \text{target concentration} \times \text{volume of distribution} \times F$$

where the target concentration in serum (ie, 400 ng/ml) was selected on the basis that it was slightly higher than therapeutic serum concentrations in humans, volume of distribution (ie, 23 L/kg) is the estimated volume of distribution reported in humans, and F (ie, 0.44) is the oral bioavailability of imipramine in humans.⁸ Both of these horses had substantial adverse effects; therefore, subsequent doses of imipramine were decreased to half the initial dose. Subsequently, pharmacokinetic studies represented results for a dose calculated at the rate of 2 mg of imipramine/kg. The 2 horses that received a dose of imipramine at a rate of 4 mg/kg were allowed a 1-month washout period before the dose calculated at a rate of 2 mg/kg was administered.

Imipramine (solution containing 25 mg/ml) was administered IV as a bolus injection during approximately a 1-minute interval. Imipramine was administered into a jugular vein of each horse, and blood samples were collected from the contralateral jugular vein, using an indwelling catheter. Catheters were thoroughly flushed with heparinized saline (0.9% NaCl) solution before each blood sample was obtained to prevent possible contamination. Blood samples were collected immediately before (time 0) and 3, 5, 10, 15, 20, 30, and 45 minutes and 1, 2, 3, 4, 6, 8, 12, and 24 hours after administration of imipramine. Blood samples were transferred into evacuated tubes. Serum was harvested by use of centrifugation, transferred into 10-ml polypropylene vials, and frozen at -20°C . Samples were shipped on dry ice via overnight carrier to the Texas Veterinary Medical Diagnostic Laboratory. On arrival, samples were stored at -20°C until time of analysis.

A CBC and serum biochemical analyses were repeated 24 hours after conclusion of the study. Results of those tests for all horses were within reference ranges.

Analysis of samples—Serum concentrations of imipramine were determined by use of **high-performance liquid chromatography (HPLC)**, using solid-phase extraction. All samples were assayed in triplicate. Five milliliters of 20 mM potassium phosphate buffer (pH 6.5) and 30 μl (0.5 μg) of acepromazine^a (5.7 mg of acepromazine maleate in 250 ml of methanol) were added to 1 ml of serum. The acepromazine served as an internal standard. Serum samples were loaded onto a preconditioned C8/benzenesulfonic acid copolymeric solid-phase extraction column^b with a 500-mg bed and a 15-ml syringe. Columns were preconditioned with 5 ml of methanol immediately followed by 5 ml of distilled water and then by 3 ml of 1.0 N HCl. After addition of samples, the column was rinsed with 5 ml of 20 mM potassium phosphate buffer (pH 6.5) followed by 3 ml of 1.0 N HCl and then by 5 ml of methanol. Residual methanol was displaced under 254 mm Hg of vacuum for 30 seconds. Samples were eluted from the column with 10 ml of a freshly prepared solution of dichloromethane:isopropanol:ammonium hydroxide (80:20:2; vol:vol); isopropanol and ammonium hydroxide were mixed prior to the addition of dichloromethane. Samples were evaporated to dryness under nitrogen gas at 45°C . The residue was suspended by addition of 150 μl of a solution of acetonitrile:distilled water (1:1), and the mixture was transferred into a HPLC vial.

The HPLC device^c was equipped with a C-18 column.^d The mobile phase was 66:34 acetonitrile:aqueous solution at an elution rate of 1 ml/min with a run time of 9 minutes. The aqueous solution contained 0.86% (vol:vol) phosphoric acid,

tetrahydrofuran, and triethylamine in HPLC-grade water. Injection volume was 50 μl , and results were monitored at a wavelength of 254 nm. Acepromazine (retention time, 5.5 minutes), desipramine (retention time, 6.8 minutes), and imipramine (retention time, 7.5 minutes) were resolved separately; each had baseline separation and did not have interfering peaks. Peak identities were confirmed by comparison of their UV spectrum with that of authenticated standards (purity reported by manufacturers, $> 99\%$). Solutions of desipramine (1 mg in 10 ml of methanol or 1 mg in 50 ml of methanol) or imipramine (1 mg in 10 ml of methanol or 1 mg in 50 ml of methanol) were used as quantitative standards.^e Calibration samples were prepared by adding 60, 100, 200, 500, 1,000, 2,000, 10,000, and 20,000 ng of imipramine or desipramine to a 16 \times 125-mm glass tube. Solvent then was evaporated to near dryness under nitrogen gas followed by addition of 1 ml of blank serum. Internal standard was added, and calibration samples were mixed thoroughly for 30 seconds prior to solid-phase extraction, as described previously.

Correlation coefficients were > 0.995 for calibration curves. Control samples were prepared for imipramine and desipramine; solutions of each were prepared at concentrations of 60, 120, and 5,000 ng/ml. Limit of quantification for desipramine and imipramine under these conditions (signal-to-noise ratio > 10 and quantitative amount within 20% of target) was 60 ng/ml. Recovery (mean \pm SD) of acepromazine, desipramine, and imipramine in horse serum was 76.9 ± 9 , 71.5 ± 12 , and $75.4 \pm 11\%$, respectively. Within-day and between-day precision was calculated, using a 1-way ANOVA and values for mean sum of squares. Mean accuracy for imipramine was 92 and 98% for the solutions containing concentrations of 120 and 5,000 ng/ml, respectively, and mean accuracy for desipramine was 93 and 101% for the solutions containing 120 and 5,000 ng/ml, respectively. Estimates of within-day **coefficient of variation (CV)** for imipramine solutions containing 120 and 5,000 ng/ml were 16.7 and 8.5%, respectively, and estimates for between-day CV were 6.9 and 6.6%, respectively. Estimates of within-day CV for desipramine in solutions containing concentrations of 120 and 5,000 ng/ml were 10.1 and 4.5%, respectively, and estimates for between-day CV were 13.1 and 2.6%, respectively.

Pharmacokinetic analysis—Concentration-versus-time data for imipramine were evaluated by use of nonlinear least-squares regression analysis. Noncompartmental analysis of the data was performed, using statistical moment theory. **Area under the plasma concentration-versus-time curve (AUC)**; calculated by use of the trapezoidal method extrapolated to infinity), **mean residence time (MRT)**, and **area under the moment curve (AUMC)** were calculated by use of a computer software program.^f **Apparent volume of distribution at steady state ($V_{d_{ss}}$)** was calculated, using the following equation: $V_{d_{ss}} = (\text{Dose} \times \text{AUMC})/(\text{AUC})^2$. **Total body clearance (Cl_T)** was calculated, using the following equation: $Cl_T = \text{Dose}/\text{AUC}$.

Results

Within a few minutes after administration, the 2 horses that received a dose of imipramine calculated at a rate of 4 mg/kg had abnormal behavior. Initial signs were severe and consisted of hyperexcitability, muscle fasciculations, hypersalivation (1 horse only), and exaggerated responses to external stimuli. Hemolysis was apparent in both horses, as evidenced by dark red-to-brown serum and urine. Results of urinalysis for 1 of these horses 3 hours after imipramine administration revealed hemoglobinuria (4+, scale of 0 to 4) and proteinuria. Although the abnormal behavior dimin-

ished in severity within 15 minutes, the horses' behavior was not completely normal for several hours.

We delayed determinations of heart rate and rhythm as well as collection of blood samples for approximately 15 minutes to avoid possible injury to horses and handlers. At that time, residual adverse effects were not observed in either of the horses.

Adverse effects observed in 3 horses administered a dose calculated at the rate of 2 mg/kg were milder and of shorter duration (maximum duration of behavioral and cardiac changes was 1 hour) than in horses that had received the higher dose. Only mild muscle fasciculations and hyperresponsiveness to sound were evident. Two horses were tachycardic (heart rate, approx 60 beats/min) at 30 minutes and 1 hour after administration of imipramine. Apparent hemolysis was detected in serum samples collected from 3 horses between 3 minutes and 3 hours after receiving the lower dose of imipramine.

Curves for mean serum concentration of imipramine-versus-time were calculated for each horse (Fig 1). After IV administration, serum imipramine concentrations in horses exceeded maximum recommended therapeutic concentrations for humans for approximately 30 minutes and remained > 50 ng/ml, the minimum therapeutic concentration in humans, for approximately 6 hours. One of the horses, the most severely affected by narcolepsy, had signs of narcolepsy 6 and 12 hours after drug administration. Interestingly, narcoleptic behavior in that horse was not detected during earlier time points, which was during the period when serum concentration of imipramine exceeded the minimum therapeutic plasma concentration. None of the other horses had signs of narcolepsy during the study period.

Pharmacokinetic data for each horse and mean values for all horses were calculated (Table 1). After IV administration of imipramine, mean MRT and Cl_T were 1.8 hours and 522 ml/kg/h, respectively. Mean Vd_{ss} approximated total body water (584 ml/kg). Desipramine, the major metabolite of imipramine in people, was not detected in any of the serum samples obtained from these horses.

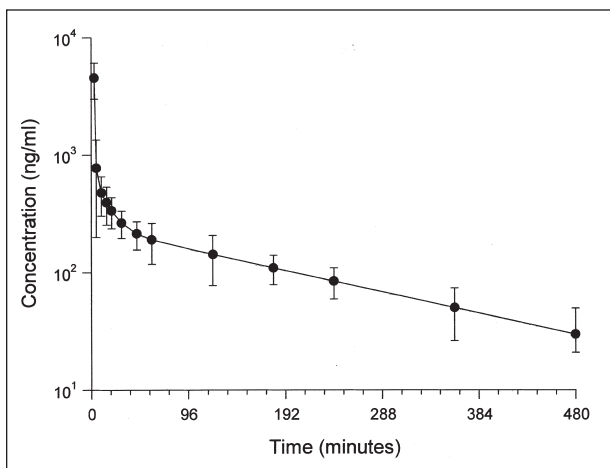


Figure 1—Mean (\pm SD) serum concentrations of imipramine versus time after IV administration of a bolus of imipramine (2 mg/kg of body weight) in 5 horses.

Table 1—Pharmacokinetic values for each of 5 horses and mean \pm SD values for all 5 horses after IV administration of imipramine (2 mg/kg of body weight)

Variable	Horse					Mean \pm SD
	1	2	3	4	5	
AUC ($h \times \mu g/ml$)	4.6	4.1	3.1	4.5	3.3	3.9 \pm 0.7
AUMC ($\mu g \times [h^2/ml]$)	566	255	143	304	176	289 \pm 167
MRT (h)	2.8	1.4	1.4	1.7	1.9	1.8 \pm 0.6
Cl_T (ml/h/kg)	432	492	654	438	600	522 \pm 102
Vd_{ss} (ml/kg)	871	514	505	492	536	584 \pm 162

AUC = Area under the plasma concentration-versus-time curve. AUMC = Area under the moment curve. MRT = Mean residence time. Cl_T = Total body clearance. Vd_{ss} = Volume of distribution at steady state.

Discussion

Disposition of imipramine after IV administration of a single dose calculated at a rate of 2 mg/kg appeared to differ in horses, compared with pharmacokinetics in humans. Although less than the value reported for humans, mean Vd_{ss} of imipramine in the horses of our study was high, consistent with the fact that imipramine is highly lipid-soluble. This suggests that imipramine should reach adequate concentrations at its presumptive site of action within the CNS. Differences in Vd_{ss} between horses and people may reflect differences in protein binding. In humans, protein binding ranges from 60 to 96%, with 4-fold interindividual variations among subjects.^{8,9} Imipramine binds with α_1 -acid glycoprotein, lipoproteins, and albumin. The degree of protein binding of imipramine in equine plasma was not determined in the study reported here.

Interestingly, desipramine, the major metabolite of imipramine in humans, was not detected in any samples obtained from these horses. Desipramine, formed by demethylation of imipramine, is an active metabolite and is believed to contribute substantially to the therapeutic efficacy of imipramine in humans.^{8,9} Other routes of metabolism for imipramine include aromatic 2-hydroxylation and dealkylation reactions. Because the assay used for our study was not designed to detect products of either of those reactions, it is not known whether they are major metabolites in horses. Mean Cl_T of imipramine in horses was less than values reported for humans.^{8,9} Although the route of elimination of imipramine in horses is unknown, it is tempting to speculate that the slower rate of clearance in horses reflected a slower rate of hepatic metabolism. The fact that desipramine was not detected in serum samples from horses suggests that another metabolic pathway exists in horses, compared with that in people. However, further studies are needed to elucidate the route of elimination of imipramine in horses. A genetic polymorphism for the oxidative metabolism of imipramine is believed to exist in humans.^{8,9} The limited variability in the values for clearance in the horses in our study (SD, 102 ml/kg/h) did not suggest that polymorphism existed within the study population.

High serum concentrations of imipramine are associated with a number of adverse effects in humans, including cardiac dysrhythmias, seizures, tremors, hypotension, and blurred vision.¹ Similar adverse effects

were evident in many of the horses in our study, particularly during the period when serum concentrations of imipramine exceeded therapeutic serum concentrations recommended for humans. An interesting effect of imipramine was the apparent induction of hemolysis in some of the horses. At high concentrations, imipramine causes lysis of erythrocytes and hepatocytes in rats by altering membrane fluidity.^{10,11} It is reasonable to assume that a similar mechanism could have resulted in lysis of erythrocytes in these horses. Because the imipramine was dissolved in sterile water for administration to the horses, hypotonic hemolysis cannot be ruled out. However, this seems less likely, because the volume of imipramine solution administered to the horses was small (approx 40 ml). Even though residual adverse effects were not detected in these horses, use of imipramine at the dosages used in this study (2 and 4 mg/kg, IV) cannot be recommended.

Efficacy of imipramine for treating narcoleptic horses in the study reported here was not evaluated because of the short duration of the study and the lack of a predictable pattern of narcoleptic attacks in the study subjects.⁷ In a case report describing repeated administration of imipramine for treatment of a horse severely affected with narcolepsy, IV administration of 250 mg resulted in a rapid response that lasted 2 to 8 hours.² Those results correspond well to the pharmacokinetic disposition of imipramine observed in the horses reported here.

Despite the fact that imipramine has been recommended for the treatment of horses with narcolepsy and other conditions,^{2,4} such administration constitutes extralabel use and represents use of a drug in a nonapproved species for a nonapproved indication. Because IV administration of a drug is required for calculation of many pharmacokinetic variables, it was necessary to administer a nonapproved formulation (filter-sterilized aqueous solution of the chemical formulation of imipramine) to the horses reported here, because a formulation is not commercially available for IV administration. Use of this nonapproved formula-

tion is not recommended for clinically affected horses because of the possibility of severe adverse reactions.

^aImipramine HCl, Sigma Chemical Co, St Louis, Mo.

^bXRDAF515 column, United Chemical Technologies, Bristol, Pa.

^cModel 1090L, Series II diode array detector, Hewlett-Packard Analytical (Aligent Technologies), Wilmington, Del.

^d5 μ m—4.6 \times 250-mm Econosphere piston, Alltech Associates, Deerfield, Ill.

^eQuantitative Quik-Chek, Alltech-Applied Science, State College, Pa.

^fPK Analyst, MicroMath, Salt Lake City, Utah.

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