Single-Dose Oral Pharmacokinetics of Pergolide Mesylate in Healthy Adult Mares*

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CLINICAL RELEVANCE

Pituitary pars intermedia dysfunction (PPID) is probably the most common disease of geriatric horses. Affected horses show a variety of clinical signs, including hirsutism, polyuria/polydipsia, immunosuppression, muscle wasting, and laminitis. The most common treatment for PPID is pergolide, a dopamine agonist; however, there are no pharmacokinetic data about the use of this drug in horses. This article describes a study designed to address this complete lack of pharmacokinetic information. The pharmacokinetics of pergolide are described in a small group of relatively young, healthy mares (n = 6), with the objective of generating data on which to base larger studies in the future. To make definitive dosing recommendations to clinicians, more studies will be needed to investigate the relationship between plasma pergolide concentrations and clinical outcomes, as well as the effect of gender, age, and concomitant disease on the absorption and disposition of this drug.

INTRODUCTION

Pituitary pars intermedia dysfunction (PPID) is probably the most common disease of geriatric horses, with an estimated prevalence ranging from 14% among aged horses in Australia to 40% of horses donated to a veterinary teaching hospital.[1,2] PPID results from dopaminergic neurodegeneration within the hypothalamus, causing increased production

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TABLE 1. Pergolide Plasma Concentrations (Individual Values, Average, and Standard Deviation) in Horses After Oral Administration of a Single Dose at 0.01 mg/kg (*n* = 6)

Time (h)	*Concentration (ng/mL)*							
	Animal 1	*Animal 2*	*Animal 3*	*Animal 4*	*Animal 5*	*Animal 6*	*Average*	*SD*
0.10	0.59	0.63	0.21	0.38	0.12	0.15	0.35	0.22
0.25	1.75	2.24	0.73	1.55	0.51	0.78	1.26	0.69
0.33	2.88	3.38	1.12	2.05	0.54	1.35	1.89	1.09
0.50	2.42	2.83	1.11	1.97	0.63	1.59	1.76	0.82
0.75	2.40	2.81	1.04	2.01	0.65	2.16	1.84	0.83
1.00	1.55	2.30	0.72	1.60	1.07	1.43	1.44	0.54
1.50	0.80	1.13	0.47	0.67	0.43	0.45	0.66	0.27
2.00	0.41	0.94	0.36	0.60	0.31	0.34	0.49	0.24
3.00	0.39	0.48	0.33	0.40	0.31	0.28	0.37	0.08
4.00	0.26	0.39	0.23	0.33	0.24	0.23	0.28	0.06
5.00	0.20	0.35	0.19	0.23	0.16	0.19	0.22	0.07
6.00	0.19	0.20	0.17	0.19	0.14	0.17	0.18	0.02
8.00	0.16	0.12	0.10	0.19	0.14	0.12	0.14	0.03
12.00	0.14	0.08	0.09	0.11	0.10	0.11	0.10	0.02
24.00	0.08	0.06	0.08	0.07	< LLOQ	0.09	0.08	0.01
48.00	< LLOQ	< LLOQ	0.06	< LLOQ	< LLOQ	0.07	0.07	0.01
72.00	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ		
96.00	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ		

LLOQ = lower limit of quantitation.

of pro-opiolipomelanocortin (POMC) peptides such as adrenocorticotropic hormone (ACTH), α-melanocyte-stimulating hormone, and β-endorphin.[3,4] Affected horses show a variety of clinical signs, including hirsutism, polyuria/polydipsia, immunosuppression, muscle wasting, and laminitis.[3,4]

Pergolide, a dopamine agonist that was first used in human medicine to treat Parkinson's disease,[5,6] has been used to treat PPID in horses for at least 2 decades.[4,7–9] Although this is a logical approach to therapy, there are no pharmacokinetic data about the use of this drug in horses. Until recently, the pharmacokinetics of pergolide in humans had not been well established because the small doses used and the extensive first-pass metabolism results in low plasma concentrations of the drug.[5] Recent developments in the field of high-performance liquid chromatography–tandem mass spectrometry have allowed for measurements of very low pergolide concentrations in plasma (pg/mL) and hence the study of pergolide pharmacokinetics in humans.[5,6,10] The objective of

this study was to provide pharmacokinetic data regarding pergolide in horses. The currently recommended dose and frequency of administration of pergolide to horses with PPID (0.00085 mg/kg q12h to 0.01 to 0.002 mg/kg q24h) are based on improvements in clinical signs and tests of pituitary function.[3,4,7–9]

MATERIALS AND METHODS

Subjects

Seven healthy adult mares were used in the study, which was approved by the Kansas State University Institutional Animal Care and Use Committee. The mares were 3 to 17 years of age; the represented breeds were Quarter horse (n = 2), Thoroughbred (n = 3), Warmblood (n = 1), and Quarter horse cross (n = 1). A pilot study was conducted in one of the animals (a 17-year-old Thoroughbred mare weighing 486 kg) to determine the ideal sampling times so that the pharmacokinetics could be accurately described. The other six horses were used in the main pharmacokinetic study. Physical findings were within normal limits. There were no clinical signs of PPID, and haircoats were normal. Results of overnight dexamethasone suppression tests were normal in all mares. Mares remained in individual stalls for the entire study period. Water, a pelleted diet, and *ad libitum*

TABLE 2. Noncompartmental Pharmacokinetic Parameters (Individual Values, Mean, Median, and Standard Error) for Pergolide Administered Orally to Horses as a Single Dose of 0.01 mg/kg (n = 6)

						AUC					
Animal ID	*Dose (mg/kg)*	λ_z *(1/h)*	$t_{½\lambda z}$ *(h)*	T_{max} *(h)*	C_{max} *(ng/mL)*	$AUC_{0-\infty}$ *(h*ng/mL)*	*%Extrapolation (%)*	V_z/F *(mL/kg)*	*CL/F (mL/[h*kg])*	$AUMC_{0-\infty}$ *(h*h*ng/mL)*	*MRT (h)*
1	0.0103	0.012	57.55	0.33	1.12	10.81	46.84	76,938.43	926.60	747.95	69.31
2	0.0096	0.051	13.61	0.33	2.88	7.87	20.45	25,148.06	1280.63	104.19	13.34
3	0.0094	0.055	12.57	0.33	2.05	7.26	18.23	25,191.74	1389.56	87.08	12.10
4	0.0096	0.060	11.49	0.33	3.38	8.03	11.76	20,861.77	1258.36	64.73	8.15
5	0.0092	0.012	60.17	0.75	2.16	13.13	48.28	66,947.66	771.22	932.29	71.90
6	0.0091	0.12	5.62	1.00	1.07	3.56	21.60	23,346.39	2881.41	24.49	7.06
Mean	0.0095	0.052	26.84	0.51	2.11	8.44	27.86	39,739.01	1417.96	326.79	30.31
SEM	0.0002	0.016	10.20	0.12	0.38	1.33	6.39	10,285.42	308.03	164.43	12.78
Median	0.0095	0.053	13.09	0.33	2.11	7.95	21.03	25,169.90	1269.50	95.64	12.72

λ_z = terminal rate constant, *AUC* = area under the time–concentration curve, *AUMC* = area under the first-moment time–concentration curve, *CL* = primary pharmacokinetic parameter of clearance, C_{max} = peak plasma concentration, *F* = unknown bioavailability, *MRT* = mean residence time, *SEM* = standard error of the mean, $t_{½\lambda}$ = terminal half-life, T_{max} = time to reach peak plasma concentration, *V* = volume of distribution

TABLE 3. Average Pharmacokinetic Parameters (Individual Values, Mean, and Standard Error) for a Three-Compartmental Model of Pergolide Pharmacokinetics Following Oral Administration to Horses as a Single Dose of 0.01 mg/kg (*n* = 6)

Parameter	*Units*	*Mean*	*SEM*
k_a	1/h	2.21	0.82
CL/F	L/h/kg	0.52	0.29
V_c/F	L/kg	1.45	0.02
CL_2/F	L/h/kg	1.30	0.52
V_2/F	L/kg	48.65	0.01
CL_3/F	L/h/kg	1.07	0.12
V_3/F	L/kg	2.11	0.10
k_{10}	1/h	0.36	0.20
k_{12}	1/h	0.90	0.36
k_{21}	1/h	0.03	0.01
k_{13}	1/h	0.74	0.08
k_{31}	1/h	0.51	0.06

CL = primary pharmacokinetic parameters of clearance, *F* = unknown bioavailability, *k* = rate constant, *SEM* = standard error of the mean, *V* = volume of distribution.

prairie grass hay were available at all times throughout the study period, except for the 8-hour period for which food was withheld before pergolide administration.

Drug Administration

A single dose of pergolide (0.01 mg/kg, Permax, Eli Lilly, Indianapolis, IN) was administered orally to each horse after an 8-hour period during which feed was withheld. This dose, which is higher than the range used clinically, was chosen primarily to ensure that plasma concentrations were high enough to adequately characterize the terminal elimination phase of the time-concentration curve. Water was available *ad libitum* to the horses throughout the study. Doses were rounded up or down to the closest 0.5 mg, as pergolide is formulated in 1-mg tablets that are easily halved. The actual dose administered ranged from 0.0091 to 0.0103 mg/kg, with a mean of 0.0095 mg/kg. Once a dose was calculated, the tablets were crushed, placed into an oral dosing syringe, mixed with molasses, and administered orally.

Pharmacokinetic Study

Pergolide was administered to six of the horses at 8:00 AM after an 8-hour fast. Blood was collected in sodium heparin blood tubes via an IV jugular catheter at 0, 0.1, 0.25, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, and 96 hours after administration of pergolide. The blood samples were immediately placed on ice. Plasma was separated from red blood cells via refrigerated centrifuge within 30 minutes of collection and was stored in appropriately labeled polypropylene tubes at –70°C until analysis.

Pergolide Analysis

Plasma concentrations of pergolide were determined with high-pressure liquid chromatography (Shimadzu Prominence, Shimadzu Scientific Instruments, Columbia, MD) and mass spectrometry (Sciex triple quadrupole, model API 4000, Applied Biosystems, Foster City, CA). The ionic transition of the ion at *m/z* 315 (parent ion $[M+H]^+$) to the ion at *m/z* 208 was monitored. Plasma samples or standards (100 µL) were added to 50 µL of internal standard (Pergolide D7, 30 ng/mL in methanol, Toronto Research Chemicals, North York, ON, Canada) and 150 µL of methanol to precipitate the proteins. The samples were vortexed for 5 seconds and centrifuged for 10 minutes at 14,000 rpm. The supernatant was transferred to a 0.45-µm centrifuge filter (Millipore, Billerica, MA) and then centrifuged for 5 minutes at

5000 rpm. The resulting extract was transferred to an injection vial with the injection volume set to 4 µL. The mobile phase consisted of (A) 0.2% acetic acid in water, and (B) 0.2% acetic acid in acetonitrile at a flow rate of 0.8 mL/min. The elution of analyte and internal standard was achieved with an isocratic mixture of 98% mobile phase B and 2% mobile phase A. Chromatography was performed using a Waters HILIC Silica 2.1-mm × 50-mm, 5-µm analytical column (Waters Milford, MA) maintained at 40°C. The standard curve was linear from 0.05 ng/mL to 20 ng/mL and was accepted if the correlation coefficient exceeded 0.99 and predicted values were within 15% of the actual values. Based on this standard curve, the upper and lower limits of quantitation (ULOQ and LLOQ) for the method were set at 20 ng/mL and 0.05 ng/mL, respectively. The accuracy of the assay was 95.3 ±7% of the actual value, and the coefficient of variation was 4% determined on 18 replicates at 0.600, 6.00, and 14.0 ng/mL.

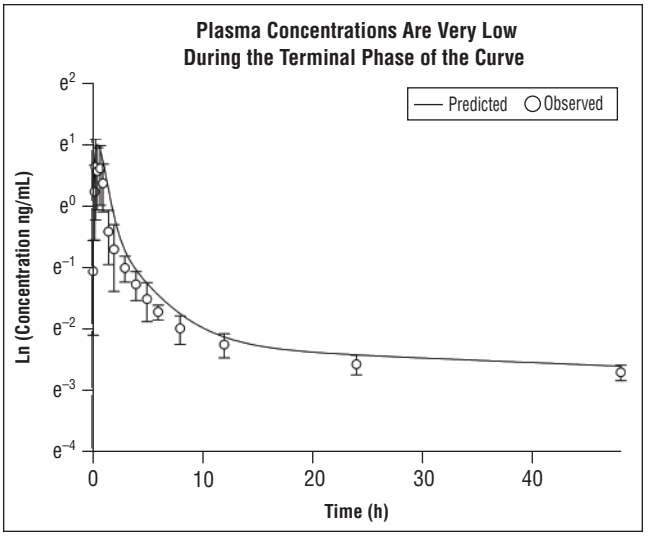

Figure 1. *Average (± SD) plasma time-concentration profile of pergolide in horses after oral administration of a single dose of 0.01 mg/kg. The solid line represents the average predicted profile based on the fitted parameters of the three-compartment open model with first-order absorption and elimination.*

Pharmacokinetic Analysis

Noncompartmental analysis of the time-concentration data was performed using WinNonlin version 5.2.1 (Pharsight Corp., Cary, NC). Parameters calculated using this method were:

- The terminal rate constant (λ_z), determined by linear regression of the ln-transformed, unweighted data. Only data points > LLOQ in the terminal portion of the time-concentration curve that fell on a straight line, based on visual inspection, were included in the linear regression analysis.
- The terminal half–life ($t_{½\lambda z}$), calculated as $0.693/\lambda_z$
- The area under the time-concentration curve (AUC) and its first moment (AUMC), using the linear trapezoidal rule with the last portion extrapolated to infinity by adding C_{last}/λ_z, where C_{last} is the last measurable concentration
- The mean residence time, calculated as AUMC/AUC
- Volume of distribution, based on the terminal phase ($Vz = Dose/\lambda_z * AUC_{0-\infty}$).

The time to reach peak plasma concentrations (T_{max}) and the peak plasma concentrations themselves (C_{max}) were observed directly from the data.

A compartmental analysis was also done us-

ing PKPlus in GastroPlus (SimulationsPlus Inc., Lancaster, CA). This program fits one-, two-, and three-compartment models to time–concentration data by directly optimizing model parameters to minimize the objective function. The data were weighted by $1/y^2$, where y is the predicted value of the data point. Choice of the best model was based on the value of Akaike's information criterion and Schwarz's criterion (lower considered to be better for both criteria). Visual inspection of observed versus predicted plots was also used to evaluate the models' goodness of fit. Parameters calculated by this method were the micro rate constants for absorption and movement between the different compartments of the three-compartment model to the average data.

Pergolide was rapidly absorbed following oral administration, with plasma concentrations reaching maximum levels (T_{max}) within 20 minutes for four horses (all within 1 hour). Maximum plasma drug concentrations (C_{max}) ranged from 1.07 to 3.38 ng/mL. The drug appeared to be widely distributed, with an average estimated value for V_z/F of approximately 40 L/kg. This is further supported by the average value for V_2/F in the three-compartment model (48.65 L/kg) and the slow rate constant for redistribution of the drug from the peripheral compartment back into the central compartment (average k_{21} = 0.03 1/h). However,

Pergolide distributes rapidly and widely in horses, resulting in very low plasma concentrations during the terminal phase of the time-concentration curve.

model (k_a and k_{10}, k_{12}, k_{21}, k_{13}, k_{31}; respectively) and the primary pharmacokinetic parameters of clearance (CL) and volume of distribution (V) for each of the compartments. Because the route of administration in this study was extravascular, the latter two parameters were corrected for an unknown bioavailability (F).

RESULTS

Time–concentration data for all the subjects are given in Table 1. Parameters of the noncompartmental model are summarized in Table 2. Decay of the average plasma time–concentration curve was triexponential, and a three-compartment open model with first-order absorption and elimination was judged to be the best for the data. The compartmental pharmacokinetic parameters are summarized in Table 3. Figure 1 illustrates the fit of the volume parameters must be interpreted with caution due to the unknown bioavailability of pergolide when administered orally to horses.

Overall, pergolide showed rapid absorption, followed by a relatively steep initial decline in concentrations related to distribution and elimination from the central compartment. Thereafter, low concentrations were sustained, with concentrations measured up to 48 hours after administration in some horses. This is most likely due to redistribution of the drug from the peripheral to the central compartment. This terminal phase was not detectable in all animals because the low concentrations were below the LLOQ in many cases. Mild depression and anorexia were noted in one mare following pergolide administration. The clinical signs were mild and resolved without treatment within 24 hours.

DISCUSSION

To our knowledge, this is the first study of the pharmacokinetics of orally administered pergolide in horses to be reported in the scientific literature. Pergolide pharmacokinetics have been studied in humans, in whom the drug was found to be rapidly absorbed (T_{max} approximately 2 to 3 hours).[6] However, plasma concentrations were 4- to 10-fold lower in humans than in horses given similar doses (0.1 to 0.9 ng/mL versus 1.1 to 3.4 ng/mL, respectively). This may be attributable to wider distribution (mean V_d/F = 14,000 L) and/or lower bioavailability in humans. In humans, pergolide undergoes extensive first-pass metabolism.[5] It is therefore important that further studies be conducted to determine the bioavailability of orally administered pergolide in horses.

The half-life of pergolide was similar in humans and horses (mean of 21 and 27 hours, respectively), although this parameter was highly variable in both species. The low plasma drug concentrations measured during the terminal part of the time–concentration curve are the most likely reason for this variability. For some horses, concentrations were still measurable at 48 hours, highlighting the slow terminal decay and resulting in the calculation of long half-lives. In other animals, concentrations were only measurable up to 24 hours, resulting in shorter calculated terminal half-lives that were more reflective of the distribution phase of the curve. More sensitive analytical methods must be developed to accurately estimate the terminal elimination half-life in all individuals.

CONCLUSION

A definitive recommendation for dosage regimens in horses is not possible based solely on the results of this study. Additional data relating plasma concentrations to clinical effect are needed. Also, this study was conducted in mares only, some of which were younger than the typical age of horses suffering from PPID. Further studies are therefore needed to determine the effect of age, gender, and disease on the pharmacokinetics of pergolide in horses.

However, we can compare the plasma concentrations in horses from this study with those considered effective for the control of Parkinson's disease in humans. In horses, plasma concentrations were 4 to 10 times higher than in humans after the same dose (0.01 mg/kg).[6] This would suggest that doses as low as 0.001 mg/kg could be effective in horses. Interestingly, the lowest dose currently used in equine clinical practice (0.002 mg/kg) is above this lower limit. In contrast, the dose originally proposed by Peters et al[7] (0.00085 mg/kg) is marginally lower than this limit, but the dose was administered twice daily, potentially resulting in some accumulation.

Finally, we learned from this study that pergolide distributes rapidly and widely in horses, resulting in very low plasma concentrations during the terminal phase of the time-concentration curve. We therefore hypothesize that shortening the dosing interval would result in better clinical outcomes by achieving and maintaining higher plasma drug concentrations. Multiple-dose studies will be needed to test this hypothesis.

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