Pituitary pars intermedia dysfunction is a common endocrinopathy in horses\(^1\) associated with ageing, in which loss of dopaminergic inhibition of melanotrophs in the pars intermedia results in increased production of pro-opiomelanocortin peptides.\(^2\) The disease is associated with the development of laminitis,\(^3,4\) a debilitating condition associated with high morbidity and mortality rates. Pituitary pars intermedia dysfunction has assumed greater importance in recent decades, as owners have shown a greater inclination to maintain horses long after retirement from previous use (eg, athletic events).\(^5\)

Treatment of horses with PPID is often indicated to reduce clinical signs associated with the disease and therefore reduce morbidity and mortality rates. Pergolide is a synthetic ergot derivate that behaves as a full agonist at dopamine D\(_2\) and D\(_3\) receptors\(^6\) and has some activity at D\(_1\) receptors.\(^7\) In horses with PPID, pergolide replaces the normal inhibitory effects of dopaminergic neurons from the hypothalamus on melanotrophs\(^8\) and is the treatment of choice for PPID.\(^2\) Intravenous infusion of dopamine receptor agonists in a horse with PPID has been found to cause a prompt decrease in plasma concentrations of pro-opiomelanocortin peptides,\(^9\) and the clinical efficacy of orally administered pergolide in horses with PPID has been reported.\(^10–14\) However, despite widespread use of pergolide, information on the pharmacokinetics of pergolide in horses is limited and dosing regimens currently are based on anecdotal experiences rather than pharmacokinetic or pharmacodynamic data. Furthermore, there is a paucity of pharmacokinetic data of ergot-derived dopamine receptor agonists in humans, and no pharmacokinetic data derived after IV administration are available for any species. A single investigation of the pharmacokinetics of pergolide after oral administration to horses has been reported.\(^15\) In that study,\(^15\) the mean half-life of pergolide was 27 hours, which is comparable with a half-life of 21 to 27 hours reported in humans.\(^16,17\) This finding is surprising because drugs that are metabolized in the liver are, in general, cleared faster in horses than in humans.\(^16\) In humans, pergolide is absorbed rapidly (reaching peak concentration within 2 to 3 hours), un-
dergoes extensive first-pass metabolism, and is eliminated completely within 4 to 5 days.¹⁹

The purpose of the study reported here was to investigate the pharmacokinetics of pergolide in horses. Specific objectives were to determine the elimination half-life (which is used to determine frequency of dose administration), volume of distribution (which is used to determine suitable starting doses), and clearance (which is used to calculate appropriate maintenance doses).

Materials and Methods

ANIMALS

Four Thoroughbred and 4 Standardbred geldings from a university research herd were used in the study. Mean ± SD age was 8 ± 3 years (range, 4 to 14 years). The day preceding the study, horses were weighed on an electronic scale specifically designed and calibrated for horses. Mean ± SD body weight was 570 ± 48 kg (range, 464 to 628 kg). All horses were considered to be in good health on the basis of results of clinical, hematologic, and blood biochemical examinations performed prior to commencement of the study. All horses had a plasma ACTH concentration < 35 pg/mL as measured with a chemiluminescent assay validated for use in horses and did not have current or previous clinical signs of PPID. Throughout the study period, horses had unlimited access to water and hay. Horses were observed continually throughout the study period, and vital parameters were assessed when abnormal behavior or clinical signs were observed. The experimental design and methods were approved by an institutional animal care and ethics committee.

EXPERIMENTAL PROCEDURES

The study was performed during early summer. Horses were stabled individually in box stalls beginning 1 day prior to commencement of the study. The neck region of each horse was aseptically prepared, lidocaine was locally administered, and a 14-gauge catheter was placed in each jugular vein. An extension set with a 1-way valve was attached to each catheter, and an injection port was placed on the end of each extension set.

PERGOLIDE ADMINISTRATION AND SAMPLE COLLECTION

On the morning of the experiment, pergolide mesylate was dissolved in dimethyl sulfoxide to create a solution with a concentration of 1 mg/mL. Each horse received a dose of 20 µg/kg (equivalent to 15.2 µg of pergolide/kg) administered IV via the catheter in the right jugular vein over a period of 10 seconds. The catheter was flushed with 20 mL of heparinized saline (0.9% NaCl) solution before and after administration of the pergolide. Blood samples for analysis were collected via the catheter in the left jugular vein. Blood samples were collected before (time 0) and 1, 2, 3, 5, 10, 15, 30, and 45 minutes and 1, 2, 3, 4, 6, 8, 10, 12, 14, 18, 24, 30, 36, 42, and 48 hours after the administration of pergolide was completed. Samples were collected into evacuated tubes containing lithium-heparin and stored on ice prior to centrifugation at 4°C; all blood samples were centrifuged within 60 minutes after collection. Plasma was separated and stored at −20°C until analysis; all plasma samples were analyzed within 2 months after collection.

DETERMINATION OF PLASMA PERGOLIDE CONCENTRATION

Investigators in another study¹⁵ identified the need for a more sensitive assay that could be used to accurately determine the terminal elimination half-life of pergolide. The LLOQ of the HPLC-MS-MS assay in that study¹⁵ was 50 pg/mL, which meant that the concentrations in some animals could not be measured beyond 24 hours after pergolide administration; thus, it was possible that calculations of terminal elimination half-life may have been more reflective of the distribution phase of the concentration-time curve. Consequently, in the present study, a more sensitive HPLC-MS-MS method was used.²⁰ Plasma samples were prepared for analysis by combining 2 mL of methanol with 1 mL of plasma. Samples were mixed in a vortexer for 5 minutes and then frozen and stored overnight at −20°C to provide more complete precipitation of protein and lipid. After thawing, samples were centrifuged at 21,500 g and transferred to autosampler vials for analysis by use of HPLC-MS-MS.²⁰ Pergolide standards (range, 0.1 to 15 ng/mL; linear range, 0.1 to 100 ng/mL) were prepared with technical-grade pergolide in methanol, and quantitation was performed on the basis of peak area.

Recovery efficiency of pergolide from plasma was determined by spiking 2 mL of native plasma with 20 µL of appropriate technical-grade pergolide standards in methanol to yield plasma concentrations of 0.2, 0.5, 1, and 5 ng/mL. These spiked plasma samples were prepared in duplicate and then processed as described previously, which resulted in a mean ± SD recovery efficiency of 83.2 ± 4.1% for all samples. The predicted LLOQ for pergolide in plasma was 8 pg/mL. Plasma was spiked with a pergolide standard to produce plasma with a concentration of 8 pg/mL. After protein precipitation with methanol, the LLOQ was determined from 6 injections of a methanol-plasma mixture to confirm the prediction. Because the relative SD was < 20%, the LLOQ was calculated as the mean + (10 X SD).

PHARMACOKINETICS

Maximum measured concentration of pergolide was determined directly from the data. Other pharmacokinetic parameters were determined for each horse by use of noncompartmental analysis with a commercial software program.⁴ The AUC₀⁻∞ and area under the first moment curve were calculated by use of the linear trapezoidal rule.²¹ Clearance was calculated as dose/AUC₀⁻∞. Mean resi-
dence time was calculated as \( \text{AUMC}_{0-\infty} / \text{AUC}_{0-\infty} \), where \( \text{AUMC}_{0-\infty} \) is the area under the first moment curve from 0 to infinity. Initial volume of distribution of the central compartment was calculated as \( \text{dose} / C_0 \), where \( C_0 \) is the concentration at time 0 estimated by extrapolation of the drug disposition curve to time 0. Terminal volume of distribution was calculated as clearance/\( \lambda_z \), where \( \lambda_z \) is the terminal elimination rate constant calculated by means of log-linear regression. Terminal elimination half-life was calculated as \((\ln 2) / \lambda_z\).

### Results

As a result of dose selection and assay sensitivity, plasma pergolide concentrations were measured until 48 hours after administration in all horses. Calculation of the terminal elimination half-life involved use of the last 8 data points for each horse. Mean \( \pm SD \) clearance of pergolide was 959 \( \pm \) 492 mL/h/kg, whereas mean elimination half-life was 5.64 \( \pm \) 2.36 hours and mean initial volume of distribution was 0.79 \( \pm \) 0.52 L/kg. Pharmacokinetic data, including nonparametric descriptive statistics, were summarized (Table 1). The pergolide concentration-time curve after IV administration was plotted (Figure 1).

Marked hyperresponsiveness, excitement (persistent walking in circles in a box stall and exaggerated responses to external visual and audible stimuli), sweating, respiratory stridor and stertor, snorting, and tachycardia were detected in 2 horses; these clinical signs peaked at 2 hours after pergolide administration. Four horses developed mild clinical abnormalities, including mild excitement, sweating, somnolence, and inappetence, and 2 horses did not have clinical signs of pergolide effects. No treatment was administered, and clinical signs of adverse effects resolved completely in all horses within 6 hours after IV administration of pergolide.

### Discussion

To our knowledge, the study reported here was the first in which the pharmacokinetics after IV administration of pergolide to horses has been described. In the present study, terminal elimination half-life of pergolide in horses (mean \( \pm SD \), 5.64 \( \pm \) 2.36 hours; range, 3.34 to 9.11 hours) was less than the half-life of 21 to 27 hours reported in humans, which presumably is a result of the greater capacity for hepatic clearance of drugs in horses, compared with that in humans. In a study of oral administration of pergolide to horses, a much longer estimate of half-life (mean, 26.84 \( \pm \) 24.97 hours; range, 5.62 to 60.17 hours) was reported.

A high dose of pergolide was also used in the present study to enable characterization of the terminal elimination phase of the concentration-time curve. In elderly human patients, pergolide concentrations up to 45 \( \mu \)g/mL have linear kinetics, which indicates saturation of metabolic pathways is not reached and that pharmacokinetic parameters obtained with high doses, such as those used in the present study, may be extrapolated to predict drug disposition in horses receiving lower doses typical of those used in clinical practice.

In pharmacokinetic studies whereby a drug is administered only orally and bioavailability is unknown, apparent volume of distribution and clearance represent theoretical values and may differ considerably from actual values. In the present study in which pergolide was administered IV, volume of distribution and clearance were actual values. Mean \( \pm SD \) terminal volume of distribution in the present study was 6.87 \( \pm \) 2.04 L/kg. Mean \( \pm SEM \) terminal volume of distribution was calculated as clearance/\( \lambda_z \), where \( \lambda_z \) is the terminal elimination rate constant calculated by means of log-linear regression. Terminal elimination half-life was calculated as \((\ln 2) / \lambda_z\).

### Table 1—Pharmacokinetic parameters of pergolide after IV administration of pergolide mesylate to 8 healthy adult horses at a dose of 20 \( \mu \)g/kg (equivalent to 15.2 \( \mu \)g of pergolide/kg).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum measured concentration (ng/mL)</td>
<td>15.62 ± 5.28</td>
<td>14.94 (8.62–24.33)</td>
</tr>
<tr>
<td>( \lambda_z ) (h(^{-1} ))</td>
<td>0.14 ± 0.06</td>
<td>0.15 (0.08–0.21)</td>
</tr>
<tr>
<td>Terminal elimination half-life (h)</td>
<td>5.64 ± 2.36</td>
<td>5.14 (3.34–9.11)</td>
</tr>
<tr>
<td>( \text{AUC}_{0-\infty} ) (ng•h/mL)</td>
<td>19.06 ± 7.68</td>
<td>18.20 (8.06–30.30)</td>
</tr>
<tr>
<td>( \text{AUC} ) extrapolated (%)</td>
<td>2.40 ± 0.76</td>
<td>2.61 (1.09–3.14)</td>
</tr>
<tr>
<td>MRT(_{0-\infty} ) (h)</td>
<td>6.14 ± 2.55</td>
<td>5.12 (3.46–10.10)</td>
</tr>
<tr>
<td>Clearance (mL/h/kg)</td>
<td>959 ± 492</td>
<td>836.50 (501–1,890)</td>
</tr>
<tr>
<td>( V_c ) (L/kg)</td>
<td>0.79 ± 0.32</td>
<td>0.72 (0.50–1.38)</td>
</tr>
<tr>
<td>( V_z ) (L/kg)</td>
<td>6.87 ± 2.04</td>
<td>7.44 (3.35–9.09)</td>
</tr>
</tbody>
</table>

\( \lambda_z \) = Terminal elimination rate constant. MRT\(_{0-\infty} \) = Mean residence time extrapolated to infinity. \( V_c \) = Initial volume of distribution (volume of distribution of the central compartment). \( V_z \) = Terminal volume of distribution (volume of distribution calculated by use of the area method).
volume of distribution reported in another study\textsuperscript{19} that involved oral administration of pergolide was 40 ± 10 L/kg, which is considerably larger than the terminal volume of distribution in the present study. Because oral bioavailability of pergolide is unknown, estimates of the volume of distribution provided in that other study\textsuperscript{19} represent the terminal volume of distribution corrected for bioavailability, which is an extremely approximate indicator of drug distribution.\textsuperscript{22} Given the capacity for hepatic clearance in horses, further investigations of the bioavailability of orally administered pergolide in horses are needed.

Pharmacokinetic parameters derived after IV administration are used to calculate dose rates. A drug administered via the IV route provides 100% of the drug systemically; however, because long-term drug administration is typically via the oral route, it is essential to know the amount of drug that is actually absorbed to adjust the dose and compensate for losses.\textsuperscript{24} The initial volume of distribution may be used to calculate appropriate loading doses, but evaluation of the mean ± SD initial volume of distribution determined in the present study (0.79 ± 0.32 L/kg) suggested that a higher starting dose for pergolide would be unnecessary; loading doses are more appropriate with drugs that have a large volume of distribution.\textsuperscript{22} This finding has important implications for the use of pergolide in horses because inappetence (the most frequent adverse effect associated with pergolide use in horses) appears to be encountered less frequently when steadily increasing doses are used to initiate treatment.\textsuperscript{25}

Calculation of an appropriate maintenance dose involves use of the following equation: maintenance dose = clearance × Css, where Css is the target effective concentration at steady state. Pergolide clearance has been elucidated, but pharmacodynamic studies are still needed to establish effective drug concentrations (the therapeutic range) for the treatment of PPID in horses.

Typical therapeutic doses of pergolide for Parkinson’s disease, a comparable degenerative condition of dopaminergic neurons in humans, are between 20 and 30 µg/kg/d, which is considerably higher than doses currently used for the treatment of PPID in horses. However, in the treatment of Parkinson’s disease in humans, initial doses are much lower and are increased gradually until a desired effect is achieved, with treatments generally being divided into 3 or 4 doses daily, to reduce adverse effects.\textsuperscript{16} Pergolide has also been used for the treatment of pituitary gland tumors, most notably prolactinomas, in humans. The mean daily doses required to control serum prolactin concentrations are approximately 1.3 and 3 µg/kg in women and men, respectively,\textsuperscript{26} which is consistent with doses typically used in horses with PPID.\textsuperscript{25} The required dose is markedly higher in human patients with macroadenomas, compared with the dose for those with microadenomas,\textsuperscript{26} which may have implications for the treatment of PPID (a condition in which a range of pathological changes are observed) and may explain the reason that responses to treatment are variable among patients.\textsuperscript{25} The variable pharmacokinetic and pharmacodynamic responses in both human and equine patients highlight the importance of monitoring clinical responses and endocrine function and titration of doses that are appropriate for each patient. Pharmacodynamic-pharmacokinetic integration is increasingly being used to guide drug dosing in both human and veterinary medicine,\textsuperscript{3} and the application of these pharmacological methods for optimization of pergolide dosing regimens will be of benefit for the management of PPID in horses.

When pergolide was first described for the treatment of PPID, the drug was administered to affected horses at dosages as high as 10 µg/kg, PO, every 24 hours\textsuperscript{26}, however, after a report\textsuperscript{19} of efficacy at a dosage of 0.85 µg/kg, PO, every 12 hours, lower doses were used.\textsuperscript{12,13} It is now widely accepted that an appropriate starting dosage is 2 µg/kg, PO, every 12 hours, with incremental increases up to 10 µg/kg if necessary.\textsuperscript{25} The favorable responses of horses with PPID to administration of low doses of pergolide administered twice daily observed in that early study\textsuperscript{19} support, as indicated by analysis of the pharmacokinetic data in the present study, that twice-daily dosing may be more appropriate than the once-daily dosing currently recommended.\textsuperscript{25} Pro-opiomelanocortin peptide production is suppressed rapidly in response to pergolide and other dopamine receptor agonists in horses with PPID, and production remains suppressed for 48 hours after oral administration of a 5-mg pergolide capsule;\textsuperscript{43} hence, there may be discordance between plasma concentrations and pharmacodynamics. The prolonged activity may be attributable to the persistence of bioactive pergolide metabolites. In rats, the sulfoxide and sulfone metabolites of pergolide have dopamine D\textsubscript{2}-receptor agonist activity similar to that of the parent molecule.\textsuperscript{29,30} Further pharmacodynamic and pharmacokinetic studies of pergolide and its metabolites are necessary to determine plasma concentrations that correlate with clinical effectiveness to determine the therapeutic range for the treatment of PPID.

In the present study, adverse effects in a number of horses were identified that coincided with high initial plasma pergolide concentrations. This finding was expected because the dose used was 10 times the amount of a typical clinical starting dose used for the treatment of PPID, and the drug was administered IV. Furthermore, healthy horses were used. Healthy human subjects have higher sensitivity to adverse effects than those afflicted with Parkinson’s disease, possibly because of a higher sensitivity of the dopaminergic system in individuals who do not have focal degeneration of dopaminergic neurons.\textsuperscript{16} The gastrointestinal, cardiovascular, and extra-pyramidal signs in horses of the present study were consistent with signs of excessive dopaminergic activity typically seen in humans.\textsuperscript{31} Nasal congestion
is also reported in healthy human subjects administered pergolide and may have been the cause of the respiratory stertor and stridor in horses of the present study; however, endoscopic examination of the nasopharynx was not performed. Inappetence is observed in 5% to 10% of horses with PPID administered standard clinical doses of pergolide, but other adverse effects are uncommon.

Limitations of the present study were the use of young horses (all geldings) that were not suspected of having PPID. This was intentional so that the population would be of comparable animals rather than a group of horses with PPID that have diversity in age, breed, body condition, and body weight. However, it will be important to establish the pharmacokinetics and pharmacodynamics of pergolide in older horses with PPID. Another limitation was the creation of a formulation of pergolide for IV administration; in the United States, there currently is no commercially available pergolide formulation for IV administration to domestic animals.

The improved sensitivity of the assay used in the present study, coupled with the higher dose used, enabled a more robust determination of pergolide pharmacokinetic parameters than has been possible previously. With an elimination half-life of approximately 6 hours, twice-daily dosing may be more appropriate than once-daily dosing to reduce peak-trough fluctuations in pergolide concentrations. The volume of distribution of pergolide is not large, which suggests that higher initial loading doses are not necessary. Further studies need to be performed to establish pergolide’s bioequivalence and pharmacodynamics, both of the parent drug and the metabolites.

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Footnotes

a. Immulite, Siemens, Bayswater, VIC, Australia.
b. Sigma-Aldrich Pty Ltd, Sydney, NSW, Australia.
d. Topfit, version 2.0, Gustav Fischer-Verlag, Jena, Germany.

References


