Pharmacokinetics and clinical effects of pirfenidone administered intravenously in horses

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Objective—To characterize the plasma pharmacokinetics and clinical effects of pirfenidone administered IV in healthy horses.

Animals—6 adult horses.

Procedures—A 15 mg/kg dose of pirfenidone was administered IV over 5 minutes. Physical variables were recorded and blood samples collected prior to infusion; 2.5 minutes after beginning infusion; at the end of infusion; and at 3, 6, 9, 12, 15, 20, 25, 30, 40, 50, 60, 75, and 90 minutes and 2, 2.5, 3, 4, 6, 8, 12, and 24 hours after completion of infusion. Plasma concentrations of pirfenidone and its metabolites were determined.

Results—Mild clinical effects, including tachycardia and muscle fasciculations, were observed during drug administration but stopped at the end of the infusion. Pirfenidone and 2 metabolites, hydroxypirfenidone and carboxypirfenidone, were detected by the end of the 5-minute infusion. Mean peak plasma concentration of pirfenidone was 182.5 µmol/L, detected at the end of the infusion. Mean peak plasma concentrations of hydroxypirfenidone and carboxypirfenidone were 1.07 and 3.4 µmol/L, respectively, at 40 minutes after infusion. No parent drug or metabolites were detected at 24 hours. Distribution of pirfenidone best fit a 2-compartment model, and the drug had mean ± SEM elimination half-life of 86.0 ± 4.7 minutes, mean body clearance of 6.54 ± 0.45 mL/kg/min, and apparent volume of distribution at steady state of 0.791 ± 0.056 L/kg.

Conclusions and Clinical Relevance—Intravenous administration of pirfenidone was tolerated with transient adverse effects during infusion, and drug clearance was rapid. (Am J Vet Res 2008;69:952–960)

Pirfenidone is a new investigational drug with unique therapeutic anti-inflammatory and antifibrotic properties. The compound is composed of a substituted pyridine (5 methyl-1-phenyl-2-[1H]-pyridone) and has a molecular weight of 185.2.1,2 It is marketed in an oral formulation in the United States and is approved for human and not veterinary use.

The drug has been investigated in humans for treatment of diverse conditions ranging from idiopathic pulmonary fibrosis to multiple sclerosis, rheumatoid arthritis, and neurofibromatosis.3,4 The oral formulation of the drug has had promising therapeutic benefits in human clinical trials and animal models for treatment of pulmonary, hepatic, cardiac, musculoskeletal, and renal fibrosis.6–11 The safety record for this medi-
and for IV administration in mice and sheep. Results of those studies indicate that the drug is rapidly distributed to peripheral tissues and metabolized in the liver and that its metabolites are eliminated primarily by the urinary tract. The principal metabolites identified include hydroxypirfenidone and carboxypirfenidone, identified in plasma and urine samples, as well as hydroxypirfenidone glucuronide and acetoxypirfenidone, identified in urine.

There have been few publications addressing the potential benefits of this medication for use in horses, although 1 study did demonstrate the ability of pirfenidone to suppress bacterial lipopolysaccharide and interleukin–1–induced nitric oxide release in an in vitro equine chondrocyte model. We are not aware of any pharmacokinetic, safety, or efficacy data available for systemic administration of this drug in horses.

Considering the current wide-ranging application of this drug in human medical practice, pirfenidone should have therapeutic potential for treatment of a variety of inflammatory conditions in horses. In particular, the safety and efficacy of pirfenidone in animal models of endotoxemia and septic shock in addition to its documented ability to reduce serum concentration of tumor necrosis factor-α and other proinflammatory cytokines and make it a promising compound for treatment of equine endotoxemia. Horses are exquisitely sensitive to endotoxin, with relatively low doses leading to severe physiologic derangements, and 10% to 40% of horses referred for treatment of acute abdominal disease have circulating endotoxins. Often these horses are unable to tolerate medications administered PO. Therefore, an IV route of administration was selected as the most appropriate for this pharmacokinetic study. Other conditions that may benefit from pirfenidone treatment in horses include intestinal ischemia reperfusion injury, chronic inflammatory airway disease, and joint disease.

On the basis of clinical experience and research in other species, we hypothesized that pirfenidone could be safely administered IV to conscious, healthy adult horses with minimal adverse physiologic effects and would be rapidly metabolized and cleared. An initial pilot study was designed to determine the maximum dose of pirfenidone that could be rapidly infused IV without severe adverse effects. The objectives of the study reported here were to determine an appropriate dose, evaluate clinical physical examination variables associated with that dose, and determine the plasma pharmacokinetic disposition of pirfenidone and its major metabolites in horses after IV administration of a single dose.

Materials and Methods

Animals—Eleven horses (5 for the pilot study and 6 for the principal study) were used. The pilot study horses consisted of 5 horses from the university research herd, including 1 Quarter Horse gelding and 4 Thoroughbreds (1 gelding and 3 mares). These horses had a mean ± SD weight of 552 ± 35.5 kg and were from 11 to 16 years old (mean, 13.4 years). Six healthy adult horses from the university research herd that had a mean ± SD weight of 566 ± 36.3 kg were used for the principal pharmacokinetic study. The horses included 3 Quarter Horses and 3 Thoroughbred geldings 11 to 15 years old (mean, 12.5 years). Horses were determined to be healthy on the basis of history and results of a complete physical examination, and they had not received any medications for > 30 days before the study began. Each horse was used once, and horses from the pilot study did not participate in the principal study.

Horses were transported to the veterinary teaching hospital on the afternoon prior to experimental testing. They were housed separately at the hospital in box stalls, had ad libitum access to water, and were fed their regular ration of alfalfa hay twice daily for the duration of the study. Body weight of each horse was recorded by use of a digital walk-on scale. The Institutional Animal Care and Use Committee of the University of California, Davis, approved the study protocol.

Instrumentation—The area over each jugular vein was clipped and infiltrated with 3 mL of a 2% solution of lidocaine hydrochloride, and a 14-gauge, 13.3-cm catheter was inserted percutaneously in each vein via standard aseptic techniques. T-port extension sets and injection adaptors were attached to the catheters to facilitate drug administration and blood sampling. The catheters and extension sets were sutured in place with 2-0 nylon. Catheter patency was maintained by flushing with 5 mL of heparinized saline (0.9% NaCl) solution (2 U/mL) every 6 hours and after each sample collection. The catheter was removed from the right jugular vein following the drug infusion, and the left jugular vein catheter was removed 24 hours after drug administration once the final blood sample was collected.

Pirfenidone—Pirfenidone for IV administration at a dose of 15 mg/kg was prepared individually for each horse from the powdered chemical form (≥ 99% pure). The 15 mg/kg dose of analytical-grade pirfenidone was made in sterile water at a concentration of 15 mg/mL (1.5% solution) at 40°C. The solution was cooled to 21°C while stirring, and sterile saline solution was added to a total treatment volume of 1 L. The pirfenidone solution was filtered through a 0.22-μm pore size filter in a laminar flow hood and transferred to an empty IV solution container for subsequent administration.

Pilot study—An initial pilot study investigation was performed to ensure that pirfenidone could be safely administered IV to horses and that pirfenidone concentration could be measured in plasma and to determine an appropriate dose to use in the principal pharmacokinetic study. Pirfenidone infusions were performed with the horses in a box stall and restrained by a handler with a halter and lead rope. The first horse received 10 mg/kg of pirfenidone, IV; 2 horses received 15 mg/kg, IV; and 2 horses received 20 mg/kg, IV. In all horses, the total infusion volume was 1 L and this dose was administered over 10 minutes. The pirfenidone solution was prepared for each horse individually as described but at the indicated dose of 10, 15, or 20 mg/kg.

Throughout the infusion, horses were under continuous observation and physical variables, including heart rate and respiratory rate, were recorded every 60 seconds. Rectal temperature was recorded at the beginning and end of the infusion period. Once the infusion was completed, horses were restrained with a handler and a halter and lead rope. The first horse received 10 mg/kg of pirfenidone, IV; 2 horses received 15 mg/kg, IV; and 2 horses received 20 mg/kg, IV. In all horses, the total infusion volume was 1 L and this dose was administered over 10 minutes. The pirfenidone solution was prepared for each horse individually as described but at the indicated dose of 10, 15, or 20 mg/kg.
were observed continuously for 60 minutes and physical variables were recorded every 15 minutes. The horses were maintained at the teaching hospital for 24 hours after the drug infusion and observed on an hourly basis during that time. For 1 horse in the pilot study that received a 15 mg/kg dose of pirfenidone, plasma samples were collected prior to the infusion; at the end of the infusion; and at 2.5, 5, 10, 15, 30, 45, 75, and 90 minutes and 2, 3, 4, 6, 8, 12, and 24 hours after infusion. Samples were stored at –20°C until analyzed for pirfenidone concentrations by use of LC-MS.

Experimental design—In the principal study, a 1-L bolus of pirfenidone (15 mg/kg) was administered through the right jugular vein catheter over 5 minutes by use of a calibrated rapid administration fluid pump and silicone tubing. Infusion and sample collection were performed in the box stall with minimal restraint consisting of a halter and lead rope. Horses were allowed free access to hay and water at all times during the study and fed 1 quart of feed concentrate during the infusion period.

Collection of blood samples and clinical evaluation—Ten milliliters of blood was collected from the right jugular vein via gentle aspiration from the catheter into 12-mL syringes and transferred directly to tubes containing sodium heparin. Samples for analysis were collected immediately before the start of the drug infusion; 2.5 minutes into the infusion; at the end of the 5-minute infusion; and 3, 6, 9, 12, 15, 20, 25, 30, 40, 50, 60, 75, and 90 minutes and 2, 2.5, 3, 4, 6, 8, 12, and 24 hours after the infusion was complete. Plasma was separated via centrifugation within 1 hour, transferred to 3-mL cryovials, and stored frozen at –20°C until analyzed.

The horses were monitored continuously during the infusion, for 3 hours after the drug was administered, and at hourly intervals until 24 hours after the drug administration was completed. Physical examination variables, including rectal temperature, pulse, and respiratory rate, were recorded prior to infusion and at every time point at which blood samples were collected. Gastrointestinal tract motility was assessed subjectively and recorded at each sample time point via auscultation of each of 4 abdominal quadrants (dorsal and ventral, left and right [30 s/site]). Horses were monitored for changes in mentation and stance (eg, excitability, apprehension, development of sedation, and appearance of discomfort).

Sample analysis—Plasma concentrations of pirfenidone and metabolites were determined by use of LC-MS analysis of precipitated plasma proteins operated under positive ion electrospray conditions with full-scan LC-MS detection of the pseudomolecular ions of pirfenidone, hydroxypirfenidone, and carboxypirfenidone. Antipyrine was used as an internal standard and molecular weight of products identified and recognized as the metabolites were determined by use of a spread sheet that included a software package for curve fitting. Models were fit by minimizing the sum of squares with the generalized reduced gradient method. A visual inspection of residual trends after fitting a single exponential equation indicated that a single compartment model would not suffice, so a 2-compartment model was used for elimination of the parent compound from the plasma. The equation of the model was as follows:

\[
C = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t}
\]

where \( C \) is the concentration at time \( t \) and \( \lambda_1 \) and \( \lambda_2 \) are exponential coefficients. \( C_1 \) and \( C_2 \) are zero-time intercepts; therefore, the zero-time concentration \( (C_0) \) was calculated as \( C_1 + C_2 \). By use of \( D \) for dose, clearance was calculated as \( CL = D/(C_1/\lambda_1 + C_2/\lambda_2) \). Initial volume of distribution \( (V) \) was calculated as \( D/CL \) or distribution volume during the terminal phase \( (V_T) \) was calculated as \( CL/\lambda_2 \) and steady state distribution volume \( (V_s) \) was calculated as \( D(C_1/\lambda_1^2 + C_2/\lambda_2^2)(C_1/CL) \). Mean residence time was calculated as \( V_s/CL \).

For pirfenidone metabolites, a single compartment model for formation and disappearance was used. The model was described by use of the equation:

\[
C = \frac{M}{Vd} \left( \frac{K_f}{K_i - K_m} \right) \left( e^{-k_m t} - e^{-k_f t} \right)
\]

where \( C \) is the concentration of the metabolite, \( t \) is time, \( K_m \) is the disappearance rate constant of the metabolite, \( K_i \) is the appearance rate constant for the formation of the metabolite, \( Vd \) is the distribution volume of the metabolite, and \( M \) is the mass of the compound. The model equation was fit to the metabolite concentration data by use of the generalized reduced gradient method as implemented in the software. The times of maximal concentration of metabolites were calculated as:

\[
t_{max} = \frac{\ln(k_f/k_m)}{k_f - k_m}
\]
The maximal concentrations (C_{max}) were obtained by substituting t_{max} into the model equation.

The variables estimated included the half-life of elimination and the area under the plasma concentration versus time curve from 0 to infinity (AUC_{ss}). Mean residence time, volume of distribution at steady state (\(V_{dss}\)), observed clearance (Cl), and the maximum observed plasma concentration (C_{max}) and the time in which it was observed (t_{max}) were calculated for each horse as well as for pooled data.

**Statistical analysis**—Statistical analysis was performed with commercial software.\(^a\) Mean ± SEM values were calculated. A repeated measures ANOVA was used to evaluate changes in clinical variables over time. Values of \(P < 0.05\) were considered significant.

**Results**

**Pilot study**—The first horse that received 10 mg of pirfenidone/kg over 10 minutes did not have any abnormal behavior or changes in mentation during or after the infusion but did have a moderately increased heart rate (from 36 to 56 beats/min) and respiratory rate (from 8 to 20 breaths/min) by 4 minutes into the infusion. These values returned to baseline immediately after the infusion was completed. The 15 mg/kg dose was also well tolerated, although both horses appeared mildly agitated and had diffuse muscle fasciculations during the infusion and both had increased heart rates (from 40 to 52 and 32 to 52 beats/min, respectively). One of the horses also had increased respiratory rate (from 12 to 32 breaths/min) during the infusion. These apparent drug-related responses were no longer evident within 10 minutes after the infusion. The 2 horses that received a 20 mg/kg dose also had generalized muscle fasciculations and appeared more excited and agitated than did the horses that had received the lower drug doses. One of the horses appeared mildly uncomfortable and pawed and chewed excessively. Both horses had increased heart rates during the drug infusion (from 16 to 56 and 36 to 60 beats/min, respectively). Both recovered rapidly and appeared clinically normal within 10 minutes of the end of the drug infusion.

Plasma samples collected from 1 horse that received 15 mg/kg had detectable pirfenidone (0.401 µg/mL) until 8 hours following infusion; drug was not detectable at 12 hours after infusion. The peak concentration was detected at the end of the 10-minute infusion (240.54 µmol/L). The drug was fitted to a nonlinear 2-phase exponential decay disposition curve with a rapid distribution phase during the first 10 minutes and a more gradual elimination phase over the next 8 hours. Data from this pilot horse were used to confirm ability to measure detectable drug concentrations after administering a 15 mg/kg dose of pirfenidone and were not included in subsequent pharmacokinetic analysis.

**Clinical observations**—There was a significant \((P = 0.018)\) change in heart rate over time for the 6 horses included in the principal study (Figure 1) but not in respiratory rate or rectal temperature. Horses had varying degrees of behavioral changes from being slightly more alert in their posture and mentation to being obviously agitated and excited. Behavioral changes included head elevation \((n = 3\) horses\), chewing \((2)\), abdominal respiratory effort \((1)\), yawning \((2)\), restlessness or agitation \((4)\), agitated pawing or kicking \((3)\), head tossing \((2)\), generalized muscle fasciculations \(\text{especially involving the triceps musculature and flank region [3]}\), and light sweat formation over the neck and abdomen \((3)\). The most severe reactions stopped immediately once the infusion was completed. Signs of agitation and excitement ceased, and heart and respiratory rates had returned to reference ranges within 10 to 15 minutes of the comple-

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**Table 1**—Mean and SE values for 2-compartment pharmacokinetic analysis after IV administration of pirfenidone (15 mg/kg) to 6 horses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(_i) (linear coefficient [mg/L])</td>
<td>18.7</td>
<td>1.35</td>
</tr>
<tr>
<td>C(_j) (linear coefficient [mg/L])</td>
<td>15</td>
<td>1.3</td>
</tr>
<tr>
<td>(\lambda_1) (exponential coefficient [1/min])</td>
<td>0.00019</td>
<td>0.00044</td>
</tr>
<tr>
<td>(\lambda_2) (exponential coefficient [1/min])</td>
<td>0.476</td>
<td>0.145</td>
</tr>
<tr>
<td>C(_o) (initial concentration [mg/L])</td>
<td>33.8</td>
<td>2.39</td>
</tr>
<tr>
<td>(V_d) (initial volume of distribution [L/kg])</td>
<td>0.456</td>
<td>0.0321</td>
</tr>
<tr>
<td>AUC (area under the plasma concentration vs time curve [(mg x min)/L])</td>
<td>2.394</td>
<td>1.79</td>
</tr>
<tr>
<td>Cl (systemic clearance [L/min/kg])</td>
<td>0.00854</td>
<td>0.00045</td>
</tr>
<tr>
<td>(V_d) (terminal volume of distribution [L/kg])</td>
<td>0.806</td>
<td>0.0595</td>
</tr>
<tr>
<td>(V_{dss}) (apparent volume of distribution at steady state [L/kg])</td>
<td>0.791</td>
<td>0.0965</td>
</tr>
<tr>
<td>MRT (mean residence time [min])</td>
<td>122</td>
<td>6.43</td>
</tr>
<tr>
<td>(t_{1/2}) (half-life of the distribution phase [min])</td>
<td>86</td>
<td>4.71</td>
</tr>
<tr>
<td>(t_{1/2}) (half-life of the elimination phase [min])</td>
<td>2.19</td>
<td>0.605</td>
</tr>
</tbody>
</table>

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**Figure 1**—Mean rectal temperature (°C), pulse (BPM), and respiratory rates (BPM) at various time points immediately prior to, during, and after rapid IV infusion of pirfenidone (15 mg/kg) to 6 horses. BPM = Beats per minute and breaths per minute.
tion of infusion. Muscle fasculation persisted for as long as 20 minutes after the infusion was completed but diminished over that time. No additional adverse reactions were detected after termination of infusion.

**Plasma pharmacokinetics**—Pirfenidone was detected in all horses in the plasma sample obtained 2.5 minutes into the infusion. After IV administration, the peak plasma concentration of pirfenidone was detected in all horses at the end of the 3-minute infusion with a mean peak of 182.5 µmol/L (range, 144.5 to 232.3 µmol/L). The apparent volume of distribution was $0.752 \pm 0.752 \text{ L/kg}$. Pharmacokinetic parameters were calculated (Table 1).

A 2-compartment model was found to best fit the data. The pirfenidone plasma concentration versus time curve was determined (Figure 2). On a semilogarithmic scale, there was a linear decrease during the first 2 hours after injection. Pirfenidone was detected at low concentration (0.09 mg/L) in only 1 of the 6 horses at 12 hours and not detected in any samples at 24 hours.

The metabolites that were consistently detectable via LC-MS analysis of the plasma samples were carboxypirfenidone and hydroxypirfenidone (Figure 3). Both metabolites were detected on the basis of molecular weight in all 6 horses by the end of the 5-minute pirfenidone infusion. On a semilogarithmic scale, there was a linear decrease during the first 2 hours after injection. Mean peak plasma values were detected for both drug metabolites at 40 minutes after infusion with mean peak hydroxypirfenidone concentration of $1.07 \pm 0.17$ µmol/L and mean peak carboxypirfenidone concentration of $3.40 \pm 0.44$ µmol/L. Hydroxypirfenidone was not detected in any samples after 6 hours, and carboxypirfenidone was not detected in any samples after 8 hours. At no time did the concentrations of hydroxypirfenidone or carboxypirfenidone exceed that of the parent compound pirfenidone.

For hydroxypirfenidone, mean $r^2$ value for fitting the 2-compartment model equation was 0.91, indicating a good fit, and this metabolite had a mean half-life of 12 hours in all 6 horses by the end of the 5-minute infusion.

![Figure 2](image1.png) Mean ± SE plasma concentrations of pirfenidone (µmol/L) at various time points after IV administration of a single dose of pirfenidone (15 mg/kg) to 6 horses.

![Figure 3](image2.png) Mean ± SE plasma concentrations of OH-pirfenidone and COOH-pirfenidone (µmol/L) at various time points after IV administration of a single dose of pirfenidone (15 mg/kg) to 6 horses.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_f$ (min$^{-1}$)</th>
<th>$K_m$ (min$^{-1}$)</th>
<th>$M/V$ (µmol/L)</th>
<th>$C_{max}$ (µmol/L)</th>
<th>$t_{max}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH-pirfenidone</td>
<td>0.0033</td>
<td>0.3445*</td>
<td>101.2</td>
<td>1.076</td>
<td>12.2</td>
</tr>
<tr>
<td>COOH-pirfenidone</td>
<td>0.0033</td>
<td>0.1689</td>
<td>172.4</td>
<td>3.418</td>
<td>23.2</td>
</tr>
</tbody>
</table>

*Significantly ($P < 0.05$) different from other compound.

$K_a$ = Appearance rate constant of metabolite, $K_d$ = Disappearance rate constant of metabolite, $M/V$ = Ratio of total mass of metabolite precursor to metabolite distribution volume, $C_{max}$ = Maximum concentration, $t_{max}$ = Time of maximum concentration. OH-pirfenidone = Hydroxypirfenidone. COOH-pirfenidone = Carboxypirfenidone.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (route)</th>
<th>Clearance (L/kg/h)</th>
<th>Vd (L/kg)</th>
<th>$t_{max}$* (min)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>40 mg/kg (IV)</td>
<td>6.0</td>
<td>0.71</td>
<td>8.6</td>
<td>34</td>
</tr>
<tr>
<td>Rat</td>
<td>125 mg/kg (PO)</td>
<td>—</td>
<td>—</td>
<td>62.4</td>
<td>31</td>
</tr>
<tr>
<td>Dog (Beagle)</td>
<td>40–400 mg/kg (PO)</td>
<td>1.8</td>
<td>—</td>
<td>16*</td>
<td>13</td>
</tr>
<tr>
<td>Sheep</td>
<td>30 mg/kg (IV)</td>
<td>1.2</td>
<td>0.71</td>
<td>24</td>
<td>14</td>
</tr>
<tr>
<td>Human adult</td>
<td>250–400 mg (PO)</td>
<td>0.5</td>
<td>—</td>
<td>62*</td>
<td>36</td>
</tr>
<tr>
<td>Human pediatric</td>
<td>250–500 mg/m$^2$ (PO)</td>
<td>85 mL/min/m$^2$</td>
<td>—</td>
<td>624</td>
<td>26</td>
</tr>
<tr>
<td>Horse</td>
<td>15 mg/kg (IV)</td>
<td>0.3924</td>
<td>0.79</td>
<td>86</td>
<td>Present study</td>
</tr>
</tbody>
</table>

*Calculated from clearance and distribution volume. *Distribution assumed to be 0.71 L/kg for calculation.

— = Not available.
of 2.01 minutes. The mean \( r^2 \) value for fitting the model for carboxypirfenidone was 0.90, also indicating a good fit, and the metabolite had a mean calculated half-life of 4.10 minutes. Other relevant pharmacokinetic variables were determined (Table 2) and compared with values obtained in mice and sheep (Table 3).

**Discussion**

The development of pirfenidone, which has documented potential for treating endotoxemia and ischemia reperfusion injuries in laboratory animals, suggested the need to determine an appropriate and safe dose for use in horses. Reports documenting the pharmacokinetics of pirfenidone in other species are available, however, pharmacokinetic variables can vary widely among species, and accurate equine pharmacokinetic data are needed before clinical trials of this medication can be designed. Results of the study reported here indicated that pirfenidone can be administered IV to conscious standing adult horses at a dose of 15 mg/kg without serious adverse effects.

The observed pharmacokinetic values corresponded with those reported for single-dose IV bolus administration of the drug in mice and sheep. The high clearance and short half-life of the parent compound and the rapid appearance of 2 major oxidative metabolites in the plasma were consistent with prompt distribution throughout the total body water and complete metabolism of the parent compound in the liver, as described in other species. The clearance rate of 0.4 L/h/kg for IV administered pirfenidone in horses was lower than that reported in mammals with a lower body weight, with a clearance of 6.0 L/h/kg reported for mice, 1.8 L/h/kg for dogs, and 1.2 L/h/kg for sheep. This is consistent with the fact that hepatic metabolism of most drugs occurs more rapidly in smaller mammals, resulting in greater clearances per unit of body weight. Data from mice and sheep also indicate that pirfenidone metabolites are 80% to 97% excreted in the urine, and although the route of drug elimination was not investigated in the present study, renal excretion of pirfenidone metabolites is considered likely in horses as well.

The distribution space of pirfenidone in horses in the present study was measured at 79% of body volume, a value similar but slightly higher than the distribution space of 71% reported in both sheep and mice. This consistency in volume of distribution of pirfenidone between species was expected because the volume of distribution per unit of body weight does not vary greatly among mammals of different sizes for most drugs. On the basis of distribution studies in other species, this volume is presumed to represent primarily body water but may also include some lipid space, particularly in brain and adipose tissue.

In the present study, as expected following IV administration, peak plasma concentrations were detected immediately after the drug was injected. Mean peak plasma pirfenidone concentration of 182.5 μmol/L was slightly higher than the peak concentrations reported in mice that were given a higher dose of pirfenidone at 40 mg/kg, IV. This difference may be attributable to different sampling time points because we were able to collect plasma samples immediately as the infusion was stopped, whereas there was a delay of approximately 1 minute before the first sample collection point in the mouse study. In the ovine pharmacokinetic study, a higher dose of 30 mg/kg, IV, was also used, and as anticipated, peak plasma concentrations were slightly higher than those detected in our equine study. Because the 15 mg/kg dose was administered over 5 minutes, whereas the dose in the ovine model was given as a bolus, a bigger difference in mean peak plasma concentration between the sheep and horses would have been expected. Plasma concentrations detected following oral administration have been found to be dose dependent, and despite rapid absorption from the gastrointestinal tract, peak concentrations were not detected immediately and did not reach those reported following IV administration in this study.

A 2-compartment model was required to describe the plasma pharmacokinetics of pirfenidone in horses in this study. This was in contrast to results reported for distribution of pirfenidone administered IV in sheep or orally in dogs in which plasma pharmacokinetics best fit a single compartment model but was similar to results reported for mice in which disappearance of parent compound from plasma followed 2-compartment elimination kinetics and the plasma concentration fell rapidly in a biexponential fashion. In 1 human clinical trial and in a rat model of pirfenidone pharmacokinetics, the authors used noncompartmental analysis. A more detailed comparison with other published pirfenidone pharmacokinetic studies is further complicated because several of the studies used repeated oral administration methods and the formulations used in those studies were not identical.

The mean terminal half-life of pirfenidone in horses was 86 minutes. Values reported in other studies have varied widely, but in previous studies of IV pirfenidone administration in mice and sheep, half-lives of 5 and 24 minutes have been reported. The longer half-life observed in horses, compared with smaller animals, is not surprising because generally, the rate of drug metabolism is related to metabolic rate and metabolic rate per kilogram of body weight is generally slower in larger animals. Half-lives reported in studies based on oral administration of the drug have been even more variable but may be less reliable because of variability in absorption and because distribution volume was estimated not calculated in those studies.

Identification of metabolites in the study reported here was presumptive and based on molecular weight of products identified and likely pathways of metabolism, including oxidation of a methyl group on the pyrildone ring followed by subsequent formation of carboxylic acid. Complete structural analysis of the metabolites was beyond the scope of the present study and may not be necessary, considering the consistency in metabolites of pirfenidone that have been identified in other mammals. The 2 principal pirfenidone metabolites detected here, hydroxypirfenidone and carboxypirfenidone, had half-lives of 2.01 and 4.10 minutes, respectively. Distribution of these compounds best fit a single compartment model. The short half-lives of the 2 principal metabolites relative to pirfenidone would...
explain why their concentrations never exceeded that of the parent compound. In contrast with the short half-life of hydroxypirfenidone in horses, the half-life in sheep was considerably longer at 44 minutes, and the plasma concentration of hydroxypirfenidone exceeded that of pirfenidone at 90 minutes after injection in that study. This difference in metabolism rates between the 2 large animal species was not anticipated, and no explanation for this difference was apparent. In the present study, the half-lives of pirfenidone and its metabolites as well as the K values of the metabolites indicated that the conversion of pirfenidone to hydroxypirfenidone is a faster process than that of the conversion to carboxypirfenidone. This relative difference of conversion rates between the principal metabolites is consistent with reported findings in sheep. However, metabolites assumed to be acetoxypirfenidone and hydroxypirfenidone glucuronide that were detected in sheep were not found in horses. This was most likely attributable to the fact that these metabolites were most consistently detected in the urine samples, and in the present study, we only evaluated pirfenidone metabolite concentrations in equine plasma samples.

A canine pharmacokinetic study investigating repeated oral administration of the drug detected significant differences among mean maximal concentrations that were related to sex, but no sex differences were observed for clearance rates. Our study was not designed to determine sex-related differences in pharmacokinetic values. Although the pilot study did include geldings and mares, the principal study from which the pharmacokinetic variables were determined was performed with geldings exclusively. Sex-associated differences in pirfenidone metabolism will require further investigation before the drug is administered to sexually intact male or female horses.

Adult horses ranging from 11 to 15 years of age, which might be considered to be middle aged, were included in this study. In 1 human clinical trial, juveniles from 3 to 19 years old were included for pharmacokinetic analysis and results were compared with adult values. Within the pediatric population in this trial, there was no evidence of age dependence in drug disposition, but somewhat higher systemic doses were required to achieve plasma concentrations comparable to adults. Potential age-related differences in distribution and metabolism would need to be required to establish dosages for neonatal equine patients.

On the basis of data extrapolated from previous studies performed in mice and sheep, a starting dose of 10 mg/kg administered over 10 minutes was chosen for the pilot study. When the first horse tolerated this dose with minimal systemic reactions, the dose was gradually increased over the course of the pilot study to 20 mg/kg administered over 10 minutes. At this higher dose, substantial systemic reactions were detected, and a dose of 15 mg/kg rapidly infused over 5 minutes was selected for the principal pharmacokinetic evaluation. Results of the pilot study also confirmed that we were able to detect the drug in equine plasma at this dose.

No serious life-threatening reactions or toxicoses were detected during the rapid IV infusion in healthy horses at 15 mg/kg. In contrast to previous studies in mice and sheep in which bolus IV injections of the drug were used, a 5-minute infusion was required to administer the 1-L volume of fluid needed to solubilize this dose of pirfenidone. This large volume was necessary because the drug tends to precipitate from solution at concentrations > 15 mg/mL. Mild to moderate signs of tachycardia, anxiety, and agitation were observed but resolved quickly on completion of the infusion. Nevertheless, we suspect that the dosage of 15 mg/kg might not be well tolerated by systemically ill horses, and future therapeutic trials should be designed with this concern in mind.

The transient and mild adverse reactions recorded during this study were consistent with those reported for other animal models. Specifically, diet-ingested pirfenidone had few systemic adverse effects on pulmonary or cardiovascular function in rats, and in long-term cardiac studies in mice and dogs, the animals had no overt signs of intolerance to the drug. In 1 study, sheep administered a bolus dose of 30 mg/kg. IV, prepared in a similar manner to that used in the present study had some initial reactions of head shaking, mild vocalizations, and unsteadiness. This lasted about 2 minutes, and no other adverse reactions were seen for the remaining 48 hours of the study. Results of a recent study suggest that the low number of adverse reactions may be attributed, at least in part, to the fact that pirfenidone suppresses inflammatory cytokine production at a post-transcriptional level.

The safety record for orally administered pirfenidone in human clinical trials has also been favorable, with relatively minor adverse reactions reported at doses ranging from 40 to 360 mg/kg/d. There have been reports of some dose-limiting toxicoses, but in most instances, patients were readministered pirfenidone at a lower dose and maintained in the clinical trials, and plasma pirfenidone concentrations did not seem correlated with adverse effects. In 1 placebo-controlled trial of pirfenidone in patients with idiopathic pulmonary fibrosis, substantial adverse events were associated with pirfenidone; however, even in this instance, adherence to the treatment regimen was similar between pirfenidone and placebo groups. The most commonly reported problems have included diarrhea, nausea, and vomiting. Other adverse effects have included skin photosensitivity, fatigue, skin rash, syncope, and dizziness. No serious toxicoses have been reported. In all of these clinical trials, most patients have not reported any adverse effects at all from taking pirfenidone orally for prolonged periods, providing additional support for future clinical trials in equine patients.

Given the rapid elimination of pirfenidone following a single bolus infusion, a continuous rate infusion of the drug will most likely be required to provide beneficial and sustained therapeutic effects in horses. The pharmacokinetic variables determined in the present study will facilitate calculation of appropriate administration rates for future therapeutic trials. Pirfenidone is also rapidly and almost completely absorbed following oral administration in other species, including dogs, humans, and rats. The decision to perform the present study by use of IV administration was made
on the basis that most clinical conditions, including endotoxemia and ischemia reperfusion, that would be likely to require treatment with this drug would also preclude the use of oral administration in horses because of gastrointestinal ileus. However, it is likely that oral administration would induce fewer adverse reactions in horses, and oral administration of pirfenidone could be beneficial for management of other inflammatory conditions, including osteoarthritis. Oral bioavailability studies would be required to determine an appropriate dose and interval for horses.

Unfortunately, a minimum therapeutic plasma concentration of pirfenidone has not been reported for any species or condition, making it difficult to decide on dose protocols for preliminary therapeutic trial in horses. In a recent human clinical trial, doses used in juvenile patients were extrapolated from adult trials in which doses that were to some extent arbitrarily selected appeared to result in good clinical results. Plasma pirfenidone concentrations in the children were evaluated in an effort to determine the pharmacokinetically comparable dose and reproduce adult drug exposure concentrations, and clinical benefits were observed in those patients. At this preliminary stage of investigating the use of this drug in horses, studies should probably be designed to maintain the highest pirfenidone plasma concentration that is well tolerated, in an effort to maximize therapeutic potential.

On the basis of observations made in the study reported here, pirfenidone can be safely administered IV to healthy conscious horses. At the dose used, the drug caused mild excitement and tachycardia during infusion in horses, but those reactions resolved quickly. Pharmacokinetic results indicated that in horses, as in other species, pirfenidone has rapid metabolism and clearance rate. Use of the drug in a form appropriate for IV administration is likely to require continuous infusion to maintain systemic concentrations adequate to treat endotoxia or other inflammatory conditions. In addition to investigating therapeutic value of the parent compound pirfenidone for endotoxemia, future studies should also be designed to investigate some of the novel analogs of the drug that are being developed to provide improved biological activity.

References


