Pharmacokinetics of phenylbutazone and its metabolite oxyphenbutazone in miniature donkeys

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Objective—To describe the pharmacokinetics of phenylbutazone and oxyphenbutazone after IV administration in miniature donkeys.

Animals—Six clinically normal miniature donkeys.

Procedure—Blood samples were collected before and 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 480 minutes after IV administration of phenylbutazone (4.4 mg/kg of body weight). Serum was analyzed in triplicate by use of high-performance liquid chromatography for determination of phenylbutazone and oxyphenbutazone concentrations. The serum concentration-time curve for each donkey was analyzed separately to estimate model-independent pharmacokinetic variables.

Results—Serum concentrations decreased rapidly after IV administration of phenylbutazone, and they reached undetectable concentrations within 4 hours. Values for mean residence time ranged from 0.5 to 3.0 hours (median, 1.1 hour), whereas total body clearance ranged from 4.2 to 7.5 ml/kg/min (mean, 5.8 ml/kg/min). Oxyphenbutazone appeared rapidly in the serum; time to peak concentration ranged from 13 to 41 minutes (mean, 26.4 minutes), and peak concentration in serum ranged from 2.8 to 4.0 mg/ml (mean, 3.5 μg/ml).

Conclusion and Clinical Relevance—Clearance of phenylbutazone in miniature donkeys after injection of a single dose (4.4 mg/kg, IV) is rapid. Compared with horses, miniature donkeys may require more frequent administration of phenylbutazone to achieve therapeutic efficacy. (Am J Vet Res 2001;62:673–675)

Phenylbutazone is a nonsteroidal anti-inflammatory drug that is used to treat horses with inflammatory conditions. The major metabolite of phenylbutazone, oxyphenbutazone, is a product of hepatic oxidative metabolism. Similar to the parent compound, oxyphenbutazone has analgesic and anti-inflammatory effects. By concurrently measuring the concentrations of parent drug and metabolite after IV administration of phenylbutazone, investigators can achieve a measure of the ability for intrinsic hepatic metabolism of that particular metabolic pathway in animals. Such an approach has been reported. In that study, the pharmacokinetics of phenylbutazone and oxyphenbutazone were determined in standard donkeys and horses.

Analysis of results of that study indicated that hepatic metabolism of phenylbutazone is more rapid in standard donkeys than in horses.

In the United States, miniature donkeys are classified on the basis of height as donkeys that are ≤14.3 cm at the most-dorsal point of the shoulder. It would appear that these miniature donkeys come from a limited genetic background, because all were initially imported into the United States from countries surrounding the Mediterranean Sea. In the clinical experiences of one author (TST), equal doses (based on body weight) of injectable anesthetic agents such as a combination of ketamine-xylazine, alone or concurrently administered along with diazepam and butorphanol, resulted in a duration of anesthesia that is shorter in miniature donkeys, compared with the duration for standard donkeys. Because each of these drugs undergoes hepatic metabolism, we hypothesized that miniature donkeys metabolize drugs more rapidly than standard donkeys, possibly requiring a shorter dosing interval for certain drugs. Differences in pharmacokinetic variables between ponies and horses, including plasma drug clearance and harmonic mean elimination half-life, have been described. Therefore, the purpose of the study reported here was to determine the pharmacokinetics of phenylbutazone and its metabolite oxyphenbutazone in miniature donkeys.

Materials and Methods

Animals—Six clinically normal castrated male miniature donkeys, ranging from 18 to 30 months old and weighing from 92 to 127 kg (mean, 110 kg), were included in the study. Miniature donkeys were housed on pasture and provided supplemental coastal Bermuda hay. On the day of the study, they were loosely tied in a covered arena. Miniature donkeys were allowed access to hay and water at all times, except during the first hour of blood collection. The study protocol was approved by an institutional laboratory animal use and care committee.

Experimental design and sample collection—Phenylbutazone (4.4 mg/kg of body weight) was administered IV during a period of approximately 1 minute. The injection was administered through a catheter inserted in a jugular vein of each miniature donkey. The catheter was thoroughly flushed with heparinized saline (0.9% NaCl) solution after drug administration and after collection of each blood sample. Blood samples were obtained before drug administration and 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 480 minutes after drug administration. Samples were transferred into evacuated tubes; serum was harvested by centrifugation and then frozen at −20°C until analyzed.

Sample analysis—Serum concentrations of phenylbutazone and oxyphenbutazone were determined by use of high-performance liquid chromatography performed in accor-
dance with the method of Peck et al. Samples were analyzed in triplicate, and mean values were used for pharmacokinetic analysis. Limit of quantification (signal-to-noise ratio > 10:1 at 240 nm, quantitative value was within 15% of target value) for phenylbutazone and oxyphenbutazone was 0.4 and 0.2 µg/ml, respectively.

Pharmacokinetics—Serum concentrations of phenylbutazone and oxyphenbutazone were plotted versus time for each miniature donkey; each graph was analyzed separately by use of a computer software program. Estimates for the following model-independent pharmacokinetic values for phenylbutazone were determined: apparent volume of distribution at steady state (Vdss), total body clearance (ClT), area under the serum concentration versus time curve (AUC, calculated by use of the trapezoidal method from time zero to infinity), and mean residence time (MRT). Estimates for time to peak serum concentration (Tpeak), peak serum concentration, and AUC for oxyphenbutazone also were determined. Values for AUC, MRT, and area under the moment curve (AUMC) were calculated by use of a computer software program. Value for Vdss was calculated by use of the following equation: Vdss = AUMC/AUC. Value for ClT was calculated by use of the following equation: ClT = Dose/AUC. Apparent volume of distribution of the central compartment (Vc) was calculated by use of the following equation:

\[ Vc = \frac{Dose}{C0} \]

where C0 is the extrapolated concentration of phenylbutazone at time 0.

Results
Mean (± SD) serum concentration of phenylbutazone was best described by a 3-compartment open model for 5 donkeys and a 2-compartment open model for 1 donkey (Fig 1), as determined solely on the basis of model-selection criteria (a modification of Akaike criteria) or lack of substantial improvement of fit when a more complex (ie, 3- or 4-compartmental) model was used. Serum concentrations of phenylbutazone decreased rapidly to undetectable amounts within 4 hours after administration. Phenylbutazone was cleared rapidly from serum, as reflected by a high ClT and short MRT (Table 1).

Mean serum concentration of oxyphenbutazone versus time curves for miniature donkeys were determined after administration of phenylbutazone (4.4 mg/kg, IV; Fig 1). Pharmacokinetic values of oxyphenbutazone also were determined for each miniature donkey (Table 2). Oxyphenbutazone quickly appeared in serum (mean Tpeak, 26 ± 11 min). Mean peak concentration of oxyphenbutazone in serum was 3.4 ± 0.51 µg/ml.

Discussion
Results of the study reported here complement and extend those of our previous study, which suggested more rapid metabolism of phenylbutazone in donkeys, compared with horses. Indeed, mean value of MRT for phenylbutazone appears even shorter in miniature donkeys (1.4 hours) than values reported for standard donkeys or horses (3.6 hours). Similarly, ClT of phenylbutazone in miniature donkeys (6 ml/kg/min) appears to be greater than for reported values in standard donkeys (2.8 ml/kg/min) or horses (0.5 ml/kg/min). Because phenylbutazone is primarily eliminated by hepatic metabolism, analysis of these results suggests that miniature donkeys have a higher rate of oxidative hepatic metabolism than standard donkeys or horses. However, studies directly comparing pharmacokinetics of phenylbutazone in standard and miniature donkeys are necessary to confirm these observations.

Concurrent measurement of oxyphenbutazone, the major metabolite of phenylbutazone, allowed calculation of pharmacokinetic values for oxyphenbutazone. The Tpeak of oxyphenbutazone was short in miniature donkeys (0.4 hours), indicating a rapid appearance of the metabolite in serum. As would be expected, Tpeak in miniature donkeys in the study reported here was shorter than values reported for

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Table 1—Pharmacokinetic variables for phenylbutazone in 6 miniature donkeys after administration of phenylbutazone (4.4 mg/kg of body weight, IV).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>Median</th>
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<tbody>
<tr>
<td>AUC (µg/ml-min)</td>
<td>586–1,037</td>
<td>752 ± 157</td>
<td>759</td>
</tr>
<tr>
<td>AUMC (µg/ml-min²)</td>
<td>20,804–105,024</td>
<td>70,824 ± 49,583</td>
<td>57,566</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>0.5–3.0</td>
<td>1.4 ± 0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Vc (ml/kg)</td>
<td>162–261</td>
<td>212.5 ± 41.9</td>
<td>213.1</td>
</tr>
<tr>
<td>Vdss (ml/kg)</td>
<td>155–1,120</td>
<td>545 ± 358</td>
<td>430</td>
</tr>
<tr>
<td>ClT (ml/kg/min)</td>
<td>4.2–7.5</td>
<td>6.0 ± 1.1</td>
<td>5.8</td>
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AUC = Area under the serum concentration versus time curve, calculated by use of the trapezoidal method from time zero to infinity. AUMC = Area under the moment curve. MRT = Mean residence time. Vc = Apparent volume of distribution of the central compartment. Vdss = Apparent volume of distribution at steady state. ClT = Total body clearance.

Table 2—Pharmacokinetics of oxyphenbutazone in 6 miniature donkeys after administration of phenylbutazone (4.4 mg/kg, IV).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Range</th>
<th>Mean ± SD</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (µg/ml-min)</td>
<td>345–1,020</td>
<td>544 ± 286</td>
<td>451</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>1.4–3.5</td>
<td>2.2 ± 0.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Vc (µg/ml)</td>
<td>2.8–4.0</td>
<td>3.4 ± 0.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Tpeak (min)</td>
<td>13–41</td>
<td>26 ± 11</td>
<td>26</td>
</tr>
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Cpeak = Peak serum concentration. Tpeak = Time to peak serum concentration.
standard donkeys (1.9 hours) or horses (6.5 hours). Maximum concentration of oxyphenbutazone is dependent on the rate of metabolism of phenylbutazone, \( V_{dss} \), of oxyphenbutazone, and rate of elimination of oxyphenbutazone. Because reliable values of \( V_{dss} \) and rate of elimination can only be determined after IV administration of a drug, these pharmacokinetic values could not be determined for oxyphenbutazone.

Although analysis of these results did not offer an apparent explanation as to why miniature donkeys metabolize drugs differently than standard donkeys or horses, several interesting possibilities exist. One explanation may be that differences in metabolic rate correlate with body surface area rather than body weight. It is possible that if the pharmacokinetic variables reported in the study reported here as well as the other aforementioned studies were expressed in terms of body surface area rather than body weight, many of the observed differences would not exist. Unfortunately, we are not aware of information in the literature regarding metabolic rate of standard donkeys or miniature donkeys. The energy costs of walking have been investigated in donkeys and were found to be lower than that of humans or cattle and ponies. However, the relationship of that variable to metabolic rate is not clear.

Another possible explanation for apparent differences in hepatic metabolic capacity of miniature donkeys, compared with that in standard donkeys and horses, would be differences in the quantity or forms of cytochrome P450 enzymes among the study populations. Interspecies and intraspecies differences in activity of cytochrome P450 enzymes have been reported.

Analysis of results of the study reported here suggests that miniature donkeys may require dosing regimens that differ from those of other equids. First, because phenylbutazone appears to be more rapidly metabolized in miniature donkeys than in standard donkeys or horses, a shorter dosing interval may be required to maintain therapeutic concentrations of phenylbutazone. However, because the primary metabolite, oxyphenbutazone, also has pharmacologic activity, recommendations cannot be made on the basis of results of this study. Furthermore, the rate of elimination of oxyphenbutazone could not be determined from results of this study. It is possible that oxyphenbutazone could be cleared more slowly in miniature donkeys, and, thus, a shorter dosing interval may be deleterious. Another concern regarding potential differences in dosing regimens between miniature donkeys and other equids is differences in oral bioavailability. Although phenylbutazone was not administered orally in this study, it is reasonable to assume that oral bioavailability of phenylbutazone would be lower in miniature donkeys than in other equids because of more rapid hepatic metabolism. Additional studies are needed to determine optimal dosing regimens for phenylbutazone in miniature donkeys.

References