A significant decrease in hepatic drug metabolism or renal function with advancing age could markedly reduce the drug elimination in aged animals and thus increase the risk of drug toxicity. For example, the elimination half-life of drugs metabolized by the cytochrome P450 (CYP) enzyme system or those excreted renally is 50% to 75% longer in human patients aged 65 years or older compared to their younger counterparts. Similarly, a significantly slower clearance (CL) of renally eliminated gentamicin was observed in aged alpacas (>14 years) compared to young adults under 4 years of age. Decreased plasma protein binding and total body water and declining liver and renal function are the most important age-related physiologic processes to affect drug distribution, metabolism, and elimination in humans. In the equine population, age-related studies are currently lacking. It is, therefore, essential to pursue targeted pharmacokinetic studies...

Comparative pharmacokinetics of phenylbutazone in healthy young-adult and geriatric horses

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OBJECTIVE
To evaluate the effects of aging on phenylbutazone (PBZ) disposition in older horses (≥25 years old) compared to young adults (4 to 10 years old) by characterizing the pharmacokinetic profile of PBZ and its active metabolite, oxyphenbutazone (OPBZ), following a 2.2-mg/kg dose, IV. We hypothesized that the disposition of PBZ will be affected by age.

ANIMALS
16 healthy horses (8 young adults aged 4 to 10 years and 8 geriatric horses ≥25 years old).

METHODS
Horses were administered a single 2.2-mg/kg PBZ dose, IV. Plasma samples were collected at designated time points and frozen at –80°C until assayed using liquid chromatography-tandem mass spectrometry. Pharmacokinetic analyses were performed using Phoenix WinNonlin, version 8.0 (Certara). Both clinical and pharmacokinetic data were compared between age groups using independent samples t tests, with P < .05 considered significant.

RESULTS
Baseline characteristics did not differ between groups, with the exception of age, weight, and plasma total solids. Plasma concentrations of PBZ were best described by a two-compartment model. The maximum plasma concentration of OPBZ was reached at 5 hours for both age groups, and the metabolite-to-parent-drug area-under-the-curve ratios were approximately 20% for both groups. None of the pharmacokinetic parameters of PBZ or its metabolite, OPBZ, differed significantly between age groups.

CLINICAL RELEVANCE
The hypothesis was rejected as there was no significant difference in PBZ disposition in young-adult horses compared to geriatric horses. Our data do not support the need for dose adjustments of PBZ in clinically healthy geriatric horses.

Keywords: equine, geriatric, phenylbutazone, pharmacokinetics, age
in geriatric horses to minimize potential adverse drug reactions and circumvent treatment failure in clinical practice.

The NSAID phenylbutazone (PBZ) is a nonselective cyclooxygenase inhibitor that is commonly used in horses for its analgesic, anti-inflammatory, and antipyretic effects. However, PBZ and other NSAIDs have been associated with ulcerogenic properties in the alimentary tract of horses and other species. Protein-losing enteropathy, localized ulcerative lesions in the right dorsal colon, gastric squamous or glandular mucosal damage, and renal crest necrosis are documented manifestations of NSAID toxicity in horses. Since risk factors of PBZ toxicity include high dose and long duration of treatment, the potential for drug toxicity is likely enhanced in geriatric horses. A recent survey found that 77% of horses > 30 years old were lame in at least 1 limb, with 97% having a reduced range of motion in at least 1 joint. A high incidence of orthopedic disease in geriatric horses thus increases the likelihood and duration of PBZ use to control inflammation and pain.

Enzyme-mediated oxidative, hydrolytic, or conjugative biotransformation in the liver produce more polar metabolites that can easily be excreted. Some of these metabolites can be pharmacologically active and enhance both the therapeutic and toxicological effects of the parent drug. The expression and activity of drug-metabolizing enzymes (i.e., CYPs) are affected by various factors, including diet, disease, species, breed, genetic polymorphisms, age, and gender. Nebbia et al previously reported that oxidative pathways, including both CYP content and NADPH cytochrome C reductase activity, were significantly lower in juvenile (up to 1 year) compared to mature or older (> 12 years) horses. In contrast to the former results, Lakritz et al previously reported no variation in liver CYP content or function in horses of increasing age (4 to 6 months, 1 year, and 2.5 to 4 years vs > 4.5 years old). However, neither study selectively evaluated geriatric animals. Studies to investigate hepatic metabolism in aged horses (over 20 to 25 years old) are necessary to determine the optimum drug dosage regimen for this population of animals.

A previous single-dose crossover study investigated the pharmacokinetics of IV and oral PBZ (4.4 mg/kg) in 6 fasted Welsh ponies (n = 3, 3 year olds; n = 3, 8 to 10 year olds). The CL of PBZ was reduced in adult compared to juvenile ponies regardless of the route of administration. Although this study did not evaluate geriatric horses, the observed changes in drug CL support the assessment of PBZ in the aged equine population. Therefore, the current study objective was to evaluate the pharmacokinetic profile of PBZ and its active metabolite, oxyphenbutazone (OPBZ), in clinically healthy horses ≥ 25 years old compared to younger adults (4 to 10 years old) following a single 2.2-mg/kg PBZ dose, IV. We hypothesized that the disposition of PBZ will be affected by age.

**Methods**

**Animals**

Sixteen client-owned horses were enrolled and divided into 2 age-based study groups (8 young-adult horses, 4 to 10 years old; 8 geriatric horses, ≥ 25 years old). The age of the animals was determined by birthdates supplied by the owners. All procedures were approved by the Institutional Animal Care and Use Committee of Cummings School of Veterinary Medicine at Tufts University. Study animals were evaluated prior to enrollment and deemed clinically healthy based on history, physical examination, complete blood count, and serum chemistry (glucose, urea, creatinine, phosphorus, calcium, magnesium, total protein, albumin, sodium, globulin, chloride, potassium, bicarbonate, bilirubin, triglycerides, alkaline phosphatase, GGT, and AST) analyses. All horses had access to ad libitum water and hay throughout the study period and were acclimated to their study environment for ≥ 1 week.

**Instrumentation and drug administration**

Horses were outfitted with 2 jugular venous catheters (Milacath Short Term; Mila International Inc) using an aseptic technique. Horses were weighed and a single 2.2-mg/kg PBZ dose (Phenylbutazone 20% Injection; Phoenix Pharmaceutical Inc) IV, was administered over 2 minutes via a dedicated jugular catheter. All catheters were flushed with 10 mL heparinized 0.9% sodium chloride solution (0.9% NaCl Injection, USP; Baxter Healthcare Corporation) using 1 mL heparin (Heparin Sodium Injection, USP; 1,000 USP units/mL; APP Pharmaceuticals LLC) per 500 mL solution after the completion of PBZ administration and every 6 hours until study completion. Blood samples (5 mL) were collected from the opposite jugular venous catheter at time (T) 0 (before administration) and 4, 8, 15, 30, and 45 minutes and 1, 2, 4, 6, 8, 12, 18, 30, and 44 hours after the completion of drug administration. Ten mL of heparinized blood were withdrawn from the catheter prior to each sample collection and subsequently returned to the subjects to prevent dilution effects. All samples were immediately placed into heparinized tubes (Vacuette NH Sodium Heparin; 6 mL; Greiner Bio-One North America Inc), chilled on wet ice, and centrifuged within 30 minutes of collection at 2,000 X g for 10 minutes. Plasma was immediately separated and stored on dry ice until transfer to a ~80°C freezer, pending analysis. An additional 2 mL of blood was taken from each animal at the 0- and 44-hour time points (T = 0; T = 44) and utilized for the comparison of PCV/total solids between the beginning and the end of the 44-hour blood collection window.
**Analysis of plasma PBZ using LC-MS-MS**

A 1-mg/mL solution of PBZ (Sigma-Aldrich) was prepared in methanol (EMD Millipore). A 10-µL aliquot of this solution was added to 90 µL of equine plasma, which had been previously analyzed to confirm the absence of PBZ, to make a solution of 100 µg/mL PBZ in plasma. From this, serial dilutions were obtained using plasma and solutions of 2 and 1 µg/mL, with 200, 40, and 8 ng/mL used as calibration standards.

Samples were prepared by adding a 50-µL aliquot of sample to 50 µL acetonitrile (which contained the internal standard OPBZ-d<sub>9</sub> [Cayman Chemical] at 2 µg/mL) to precipitate proteins. These mixtures were vortexed for 10 seconds and centrifuged at 16,162 X g for 10 minutes. Forty µL acetonitrile was added to a 40-µL aliquot of the supernatant of each sample, and these mixtures were again vortexed and centrifuged for 10 minutes at 16,162 X g. The supernatants of this step were used for injections (2 µL) into the HPLC system. Standards and samples were prepared in 2 steps as this resulted in clearer solutions that prevented increasing pressure in the HPLC system. This preparation was used for samples collected at 44 hours, these samples were analyzed for PBZ, relative to PBZ-d<sub>9</sub> (Cayman Chemical) at 400 µg/mL) to precipitate proteins. These mixtures were vortexed for 10 seconds and centrifuged at 16,162 X g for 10 minutes. Subsequently, 50 µL acetonitrile was added to a 50-µL aliquot of each supernatant, and these mixtures were again vortexed and centrifuged for 10 minutes at 16,162 X g. The supernatants of this step were used for injection (2 µL) into the HPLC system.

The analytical system consisted of an Agilent 1100 HPLC system coupled to an AB/SCIEX API4000 mass spectrometer for detection. The column was a Kinetex PFP Column (100 X 2.1 mm, 2.6 µm particles, 100 Å pores; Phenomenex), and the mobile phase was water:acetonitrile (55:45, v/v) with 0.1% formic acid. The mobile phase was pumped at 250 µL/min. The transitions monitored with the mass spectrometer in positive ion mode were 325.2/160.2 for OPBZ and 334.4/169.4 for OPBZ-d<sub>9</sub>. For OPBZ, intraday assay RSDs were 0.63% to 3.40% (n = 3), and interday assay RSDs were 0.72% to 8.37% (n = 3). The accuracy of the assay for PBZ and OPBZ ranged from 97.5% to 100.7% and 93.3% to 98.4%, respectively. The LLOQ for PBZ was 4 ng/mL, and the LOD was 1 ng/mL.

**Pharmacokinetic analyses**

Compartamental and noncompartamental analyses of plasma PBZ concentrations were performed using pharmacokinetic software (Phoenix WinNonlin, version 8.0; Certara). The distribution half-life (t<sub>1/2</sub>) and elimination t<sub>1/2</sub> phases were calculated using the two-compartment first order infusion equation (C<sub>t</sub> = A<sub>τ</sub>e<sup>-αt</sup> + B<sub>τ</sub>e<sup>-βt</sup>), where α and β are the hybrid rate constants, R is the infusion rate in mg/h, and the values of A<sub>τ</sub> and B<sub>τ</sub> are the extrapolated concentrations to time 0 of the distribution and elimination phases. The area under the plasma concentration time curve (AUC) from time 0 to infinity (AUC<sub>0→∞</sub>) and the area under the first moment curve from time 0 to infinity (AUMC<sub>0→∞</sub>) were determined using the trapezoidal method with extrapolation to infinity. The systemic CL was determined using the dose/AUC, and the mean residence time (MRT) was obtained from the ratio of AUMC<sub>0→∞</sub> to AUC<sub>0→∞</sub>. The volume of distribution of the central compartment (V<sub>c</sub>) was determined from the dose/A + B, and the steady-state volume of distribution was calculated as V<sub>ss</sub> = CL<sub>c</sub>/MRT. The plasma concentrations of OPBZ were analyzed using noncompartamental analysis (Phoenix WinNonlin; Certara), and the following pharmacokinetic parameters were calculated for OPBZ: AUC<sub>0→∞</sub> and metabolite elimination rate constant (λ<sub>me</sub>) and its t<sub>1/2</sub>. The time to maximum plasma concentration was to 90 µL of equine plasma, which had been previously analyzed to confirm the absence of OPBZ, to make a solution of 100 µg/mL OPBZ in plasma. From this, serial dilutions were obtained using plasma and solutions of 2 and 1 µg/mL, with 200, 40, and 8 ng/mL used as calibration standards.

**Analysis of plasma OBPZ using LC-MS-MS**

A 1-ng/mL solution of OPBZ (Sigma-Aldrich) was prepared in methanol. A 10-µL aliquot of this solution was added to 90 µL of equine plasma, which had been previously analyzed to confirm the absence of OPBZ, to make a solution of 100 µg/mL OPBZ in plasma. From this, serial dilutions were obtained using plasma and solutions of 2 and 1 µg/mL, with 200, 40, and 8 ng/mL used as calibration standards.

Samples were prepared by adding a 50-µL aliquot of sample to 50 µL acetonitrile (which contained the internal standard OPBZ-d<sub>9</sub> [Cayman Chemical] at 2 µg/mL) to precipitate proteins. These mixtures were vortexed for 10 seconds and centrifuged at 16,162 X g for 10 minutes. Subsequently, 50 µL acetonitrile was added to a 50-µL aliquot of each supernatant, and these mixtures were again vortexed and centrifuged for 10 minutes at 16,162 X g. The supernatants of this step were used for injection (2 µL) into the HPLC system.

The analytical system consisted of an Agilent 1100 HPLC system coupled to an AB/SCIEX API4000 mass spectrometer for detection. The column was a Kinetex PFP Column (100 X 2.1 mm, 2.6 µm particles, 100 Å pores; Phenomenex), and the mobile phase was water:acetonitrile (55:45, v/v) with 0.1% formic acid. The mobile phase was pumped at 250 µL/min. The transitions monitored with the mass spectrometer in positive ion mode were 325.2/160.2 for OPBZ and 334.4/169.4 for OPBZ-d<sub>9</sub>. For OPBZ, intraday assay RSDs were 0.63% to 3.40% (n = 3), and interday assay RSDs were 0.72% to 8.37% (n = 3). The accuracy of the assay for PBZ and OPBZ ranged from 97.5% to 100.7% and 93.3% to 98.4%, respectively. The LLOQ for PBZ was 4 ng/mL, and the LOD was 1 ng/mL.

**Pharmacokinetic analyses**

Compartamental and noncompartamental analyses of plasma PBZ concentrations were performed using pharmacokinetic software (Phoenix WinNonlin, version 8.0; Certara). The distribution half-life (t<sub>1/2</sub>) and elimination t<sub>1/2</sub> phases were calculated using the two-compartment first order infusion equation (C<sub>t</sub> = A<sub>τ</sub>e<sup>-αt</sup> + B<sub>τ</sub>e<sup>-βt</sup>), where α and β are the hybrid rate constants, R is the infusion rate in mg/h, and the values of A<sub>τ</sub> and B<sub>τ</sub> are the extrapolated concentrations to time 0 of the distribution and elimination phases. The area under the plasma concentration time curve (AUC) from time 0 to infinity (AUC<sub>0→∞</sub>) and the area under the first moment curve from time 0 to infinity (AUMC<sub>0→∞</sub>) were determined using the trapezoidal method with extrapolation to infinity. The systemic CL was determined using the dose/AUC, and the mean residence time (MRT) was obtained from the ratio of AUMC<sub>0→∞</sub> to AUC<sub>0→∞</sub>. The volume of distribution of the central compartment (V<sub>c</sub>) was determined from the dose/A + B, and the steady-state volume of distribution was calculated as V<sub>ss</sub> = CL<sub>c</sub>/MRT. The plasma concentrations of OPBZ were analyzed using noncompartamental analysis (Phoenix WinNonlin; Certara), and the following pharmacokinetic parameters were calculated for OPBZ: AUC<sub>0→∞</sub> and metabolite elimination rate constant (λ<sub>me</sub>) and its t<sub>1/2</sub>. The time to maximum plasma concentration was to 90 µL of equine plasma, which had been previously analyzed to confirm the absence of OPBZ, to make a solution of 100 µg/mL OPBZ in plasma. From this, serial dilutions were obtained using plasma and solutions of 2 and 1 µg/mL, with 200, 40, and 8 ng/mL used as calibration standards.

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Coefficients of variation, the lowest sum of squares, by a 2-compartment model as determined by the individual plasma concentrations were best described.

The average plasma concentration of 2.2 mg/kg PBZ, IV, in 8 young-adult and 8 aged horses are represented of 2.2 mg/kg PBZ, IV, in 8 young-adult and 8 aged horses are represented.

Horses were represented of 2.2 mg/kg PBZ, IV, in 8 young-adult and 8 aged horses are represented.

The macokinetic parameters following the administration of 2.2 mg/kg PBZ, IV, in 8 young-adult and 8 aged horses are represented (Table 1).

The plasma concentration-time curves and pharmacokinetic parameters following the administration of 2.2 mg/kg PBZ, IV, in 8 young-adult and 8 aged horses are represented (Table 2; Figure 1).

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Baseline pharmacokinetic characteristics did not differ between the 2 animal groups.

Statistical analysis

GraphPad Prism (version 7) was used for statistical analyses. Comparisons between 2 animal groups were performed using independent samples t tests, with P < .05 considered statistically significant. Data normality was confirmed using the Shapiro-Wilk test.

Results

Baseline characteristics did not differ between older and young-adult horses, with the exception of age, body weight, and serum total solids (Table 1). The older age group was composed of horses with a mean age of 28.3 years (25 to 34 years), whereas the mean age of the young-adult group was 7.3 years (4 to 10 years). The mean body weight of the older horses (451 kg; range, 389 to 514) was significantly lower than that of the young adults (508 kg; range, 432 to 568; P = .02). Additionally, mean serum total solids were higher in older animals (7.2 g/dL; range, 6.4 to 7.8 g/dL) compared to the young-adult group (6.5 g/dL; range, 6.0 to 7.2 g/dL; P = .02). Neither PCV nor total solids differed significantly over time (T0 vs T44 hours) in either age group. All horses remained clinically healthy without evidence of adverse drug reactions or documented complications during or subsequent to the study period.

The plasma concentration-time curves and pharmacokinetic parameters following the administration of 2.2 mg/kg PBZ, IV, in 8 young-adult and 8 aged horses are represented (Table 2; Figure 1). The individual plasma concentrations were best described by a 2-compartment model as determined by the coefficients of variation, the lowest sum of squares, T_max and maximum plasma concentration C_max were observed from the data. The fraction metabolized was calculated using the ratio of the plasma AUC_0–∞ of PBZ for each horse in both groups.

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The plasma concentration-time curves and pharmacokinetic parameters following the administration of 2.2 mg/kg PBZ, IV, in 8 young-adult and 8 aged horses are represented (Table 2; Figure 1).

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Table 1—The baseline characteristics of the 16 horses enrolled in the pharmacokinetics study.

<table>
<thead>
<tr>
<th>PK parameters</th>
<th>Young-adult horses (N = 8)</th>
<th>Geriatric horses (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>7.3 ± 1.9 (4–10)</td>
<td>28.3 ± 3.3 (25–34)*</td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Breed</td>
<td>Dutch Warmblood</td>
<td>Arabian</td>
</tr>
<tr>
<td></td>
<td>Morgan</td>
<td>Morgan</td>
</tr>
<tr>
<td></td>
<td>Lusitano</td>
<td>Paint</td>
</tr>
<tr>
<td></td>
<td>Quarter Horse</td>
<td>Quarter Horse</td>
</tr>
<tr>
<td></td>
<td>Warmblood</td>
<td>Trakehner cross</td>
</tr>
<tr>
<td>BCS (1–9)</td>
<td>6 (5–7.5)</td>
<td>5.25 (4.5–6.5)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>508 ± 39 (432–568)*</td>
<td>451 ± 49 (389–514)*</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.5 ± 0.3 (37.2–38.1)</td>
<td>37.2 ± 0.5 (36.1–37.6)</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>35 ± 3 (30–38)</td>
<td>36 ± 3.5 (30–42)</td>
</tr>
<tr>
<td>RR (breaths/min)</td>
<td>11 ± 1 (9–12)</td>
<td>11 ± 1.5 (8–12)</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>36 ± 4 (32–41)</td>
<td>38 ± 3 (34–42)</td>
</tr>
<tr>
<td>Total solids (g/dL)</td>
<td>6.5 ± 0.5 (6–7.2)*</td>
<td>7.2 ± 0.5 (6.4–7.8)*</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.2 ± 0.3</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>Globulin (g/dL)</td>
<td>3.2 ± 0.5</td>
<td>3.7 ± 0.6</td>
</tr>
</tbody>
</table>

Table 2—Pharmacokinetic (PK) parameters of phenylbutazone following a single 2.2-mg/kg dose, IV, in healthy young-adult and geriatric horses (n = 8 per group).

<table>
<thead>
<tr>
<th>PK parameters</th>
<th>Young-adult horses</th>
<th>Geriatric horses</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (μg/ml)</td>
<td>15.36 ± 1.82</td>
<td>15.16 ± 2.27</td>
</tr>
<tr>
<td>α (h^-1)</td>
<td>1.08 ± 0.39</td>
<td>1.07 ± 0.57</td>
</tr>
<tr>
<td>B (μg/ml)</td>
<td>9.17 ± 2.12</td>
<td>7.77 ± 2.40</td>
</tr>
<tr>
<td>β (h^-1)</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>C_max (μg/ml)</td>
<td>24.24 ± 2.89</td>
<td>22.64 ± 3.26</td>
</tr>
<tr>
<td>t_1/2α (h)</td>
<td>0.77 ± 0.46</td>
<td>0.88 ± 0.59</td>
</tr>
<tr>
<td>t_1/2β (h)</td>
<td>0.64 ± 0.23^a</td>
<td>0.65 ± 0.36^a</td>
</tr>
<tr>
<td>AUC_0–∞ (μg*h/mL)</td>
<td>96.56 ± 18.31</td>
<td>82.32 ± 16.18</td>
</tr>
<tr>
<td>AUMC_0–∞ (μg*h^2/mL)</td>
<td>726.97 ± 231.19</td>
<td>559.94 ± 216.68</td>
</tr>
<tr>
<td>CL (L/h/kg)</td>
<td>0.02 ± 0.04</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>7.37 ± 0.98</td>
<td>6.61 ± 1.23</td>
</tr>
<tr>
<td>V_1 (L/kg)</td>
<td>0.17 ± 0.02</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>V_c (L/kg)</td>
<td>0.09 ± 0.01</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>V_d (L/kg)</td>
<td>0.08 ± 0.02</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>k_12 (h^-1)</td>
<td>0.26 ± 0.04</td>
<td>0.29 ± 0.06</td>
</tr>
<tr>
<td>k_12 (h^-1)</td>
<td>0.45 ± 0.21</td>
<td>0.44 ± 0.31</td>
</tr>
<tr>
<td>k_21 (h^-1)</td>
<td>0.49 ± 0.17</td>
<td>0.47 ± 0.26</td>
</tr>
</tbody>
</table>

Data are reported as mean ± SD with the exception of half-life harmonic mean ± pseudo SD. There was no statistical difference between young-adult and geriatric groups for any of the pharmacokinetic parameters (P > .05).

A = Extrapolated concentrations to time 0 of the distribution phase. α = Rate constant of distribution phase. AUC_0–∞ = Area under the plasma concentration time curve from time 0 to infinity. AUMC_0–∞ = Area under the first moment curve from time 0 to infinity. CL = Systemic clearance. C_max = Maximum plasma concentration of the drug at the end of infusion. MRT = Mean residence time. t_1/2α = Distribution half-life. t_1/2β = Elimination half-life. V_c = Volume of distribution in the central compartment. V_d = Volume of distribution in the central compartment. V_1 = Volume of distribution during the elimination phase. V_10 = Volume of distribution at steady state.

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Figure 1—The mean plasma phenylbutazone (PBZ) concentration-versus-time curve (± SD) in 8 young-adult and 8 geriatric horses following a single 2.2-mg/kg PBZ dose, IV. The insert shows the mean plasma concentration-time profile (± SD) for the first 4 hours postdose.

Figure 2—Mean observed (± SD) and predicted plasma concentrations of PBZ for healthy young-adult horses following a single 2.2-mg/kg PBZ dose, IV. Conc = Concentration. PBZ = plasma phenylbutazone.
Figure 3—Mean observed (± SD) and predicted plasma concentrations of PBZ for healthy geriatric horses following a single 2.2-mg/kg PBZ dose, IV. Conc = Concentration. PBZ = plasma phenylbutazone.

Figure 4—The mean plasma oxyphenbutazone (OPBZ; an active metabolite of PBZ) concentration-versus-time curve (± SD) in 8 young-adult and 8 geriatric horses following a single 2.2-mg/kg PBZ dose, IV. PBZ = plasma phenylbutazone.
and the Akaike criterion of the estimated parameters. The mean predicted-versus-observed data of the plasma concentrations of PBZ for the age groups are represented (Figures 2 and 3) for young-adult and geriatric animals, respectively. None of the PBZ pharmacokinetic parameters, including mean maximum plasma concentration (22.64 ± 3.26 µg/mL vs. 24.24 ± 2.89 µg/mL; \( P = 0.32 \)) or distribution (0.88 ± 0.59 hours and 0.77 ± 0.46 hours; \( P = 0.69 \)) and elimination (5.66 ± 0.66 hours and 6.03 ± 0.69 hours; \( P = 0.29 \)) half-lives, significantly differed between geriatric and young-adult horses, respectively. Similarly, the pharmacokinetic parameters were not significantly different between groups for the metabolite (Figure 4; Table 3). The maximum plasma concentration of OPBZ was reached at approximately 5 hours for both age groups, and elimination of the metabolite did not differ. The AUC∞ of OPBZ represented approximately 16% of the total of PBZ AUC∞ in plasma in young-adult horses and 21% in geriatric horses.

Table 3—Pharmacokinetic (PK) parameters of the metabolite, OPBZ, following a single 2.2-mg/kg phenylbutazone dose, IV, in healthy young-adult and geriatric horses (n = 8 per group).

<table>
<thead>
<tr>
<th>PK parameters</th>
<th>Young-adult horses</th>
<th>Geriatric horses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (µg/mL)</td>
<td>0.89 ± 0.24</td>
<td>1.05 ± 0.25</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>5.0 ± 1.1</td>
<td>5.0 ± 2.14</td>
</tr>
<tr>
<td>λz = λm</td>
<td>0.095 ± 0.014</td>
<td>0.098 ± 0.015</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>7.37 ± 0.94</td>
<td>7.12 ± 1.14</td>
</tr>
<tr>
<td>zme</td>
<td>7.26(^a)</td>
<td>6.96(^a)</td>
</tr>
<tr>
<td>AUC∞ (µg*h/mL)</td>
<td>15.25 ± 4.43</td>
<td>16.94 ± 4.53</td>
</tr>
<tr>
<td>f(m)</td>
<td>0.16 ± 0.23</td>
<td>0.21 ± 0.28</td>
</tr>
</tbody>
</table>

Data are reported as mean ± SD with the exception of half-life harmonic mean. There was no statistical difference between young-adult and geriatric groups for any of the pharmacokinetic parameters \( P > 0.05 \).

\( \text{AUC}_{\infty} \) = Area under the plasma concentration time curve from 0 to infinity. \( C_{\text{max}} \) = Maximum plasma concentration of the drug after administration. \( f_{\text{m}} \) = Fraction metabolized. \( \lambda_{\text{m}} \) = Metabolite elimination rate constant. \( t_{1/2} \) = Half-life of metabolite elimination. \( t_{\text{max}} \) = Time to maximum plasma concentration.

**Discussion**

This is the first study to investigate PBZ pharmacokinetics in geriatric horses to determine whether dose adjustments are indicated in aged horses to decrease the potential risk of drug toxicity. Geriatric animals represent approximately 11.4% of US horses based on a 2015 survey\(^18\) and thus account for a substantial proportion of all large-animal veterinary patients. Mauderly and Hahn\(^19\) suggest that a 25-year-old horse corresponds in age-related changes to a 71-year-old human. Since physiologically age-related changes that affect drug pharmacokinetics occur in humans at ages over 65 and most notably after 70 years, a geriatric equine population ≥ 25 years old was chosen for this study.

A two-compartment model was fitted to the plasma concentrations of PBZ, which agrees with previous reports.\(^20\) The pharmacokinetic parameters estimated in the current study for young adults were consistent with those documented previously.\(^14,20-22\)

For example, Lees et al\(^14\) reported a mean elimination half-life of 5.54 hours and clearance of 0.019 L/h/kg for adult ponies (8 to 10 years) compared to 6.03 hours and 0.023 L/h/kg in our study, respectively. Furthermore, there were no significant differences in our results between young-adult and geriatric horses. Similarly, Della Rocca et al\(^23\) also found no differences in naproxen disposition in older horses (19 and 26 years) when results were compared to the Cagnardi et al\(^24\) study of young horses (aged 2 to 8 years).

The elimination of PBZ is dependent principally on hepatic metabolism. Of the identified metabolites, OPBZ and \( \gamma \)-hydroxyphenylbutazone have been described as pharmacologically active and predominant over other metabolites in plasma.\(^24\) Alterations in hepatic CYP activity in geriatric horses might thus decrease PBZ elimination and result in drug accumulation. The binding of PBZ to plasma proteins (primarily albumin) in aged horses is ≥ 98%.\(^25\) A reduction in plasma albumin concentration could theoretically potentiate the risk for NSAID-related toxicity by increasing the concentration of pharmacologically active (nonbound) compound in horses with altered hepatic metabolism. Although age-related decreases in albumin concentrations are commonly reported in elderly people,\(^26\) similar observations have not been documented in horses.\(^27,28\) However, Ralston et al\(^28\) found an increase in serum globulins (4.3 g/dL vs. 3.6 g/dL) in older (≥ 20 years) compared to younger (< 5 years) animals. In contrast, neither baseline albumin nor globulin concentrations statistically differed between age groups in the current report.

In the current study, we analyzed OPBZ plasma concentrations, as a principal metabolite of PBZ, for both groups. The maximum concentration of OPBZ (0.89 ± 0.24 and 1.05 ± 0.25 µg/mL for young and geriatric horses, respectively) was reached at 5 hours in both groups, suggesting no change in metabolite formation rate. The mean rate constant of metabolite elimination was not statistically different between age groups (young adult, 0.095 ± 0.014 h\(^{-1}\); geriatric, 0.098 ± 0.015 h\(^{-1}\)). The ratio of the AUC of the metabolite to the parent drug was approximately 20% for both groups.

This is in agreement with a previous study in which Lees et al\(^10\) reported an OPBZ AUC from time 0 to 72 hours of 244.5 ± 7.8 µg*h/mL and a PBZ AUC from time 0 to 72 hours of 35.2 ± 1.6 µg*h/mL (approx 14% OPBZ) when 4.4 mg/kg PBZ was given IV to 3 ponies. There was no statistically significant difference in any of the pharmacokinetic parameters of the metabolite concentrations between young-adult and geriatric horses.

In conclusion, there was no statistically significant difference in the disposition of PBZ at a 2.2-mg/kg dose, IV, in geriatric compared to young-adult healthy horses. The current FDA-approved equine dose of IV PBZ is 1 to 2 g/454 kg (2.2 to 4.4 mg/kg) for no more than 5 days.\(^29\) Based on our findings, dose adjustments are not required for healthy geriatric horses.
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Disclosures

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References


