Pharmacokinetics of Orally Administered Phenylbutazone in African and Asian Elephants (Loxodonta africana and Elephas maximus)

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PHARMACOKINETICS OF ORALLY ADMINISTERED PHENYLBUTAZONE IN AFRICAN AND ASIAN ELEPHANTS (*LOXODONTA AFRICANA* AND *ELEPHAS MAXIMUS*)

Ursula Bechert, D.V.M., Ph.D., J. Mark Christensen, Ph.D., C. Nguyen, Ph.D., R. Neelkant, Ph.D., and E. Bendas, Ph.D.

Abstract: The pharmacokinetic parameters of phenylbutazone were determined in 18 elephants (*Loxodonta africana* and *Elephas maximus*) after single-dose oral administration of 2, 3, and 4 mg/kg phenylbutazone, as well as multiple-dose administrations with a 4-wk washout period between trials. After administration of 2 mg/kg phenylbutazone, mean serum concentrations peaked in approximately 7.5 hr at 4.3 ± 2.02 μg/ml and 9.7 hr at 7.1 ± 2.36 μg/ml for African and Asian elephants, respectively, while 3 mg/kg dosages resulted in peak serum concentrations of 7.2 ± 4.06 μg/ml in 8.4 hr and 12.1 ± 3.13 μg/ml in 14 hr. The harmonic mean half-life was long, ranging between 13 and 15 hr and 39 and 45 hr for African and Asian elephants, respectively. There was evidence of enterohepatic cycling of phenylbutazone in Asian elephants. Significant differences ($P < 0.0001$) in pharmacokinetic values occurred between African and Asian elephants for clearance (27.9 and 7.6 ml/hr/kg, respectively), terminal half-life (15.0 and 38.7 hr, respectively), and mean residence time (22.5 and 55.5 hr, respectively) using 2-mg/kg dosages as an example. This suggests that different treatment regimens for Asian and African elephants should be used. There were no apparent gender differences in these parameters for either elephant species.

Key words: Phenylbutazone, pharmacokinetics, African elephants, Asian elephants, NSAID.

INTRODUCTION

There is a high prevalence of musculoskeletal disorders (e.g., trauma, arthritis) among captive elephants (*Loxodonta africana* and *Elephas maximus*) reported to occur in more than 70% of the population in North America in a retrospective study.[30] To treat these and other conditions, nonsteroidal anti-inflammatory agents (NSAIDS), such as phenylbutazone, are used strictly on an empirical basis in elephants. However, results from previous studies with other drugs[1,18] suggest that metabolic scaling calculations for elephants are unreliable,[27] either under-[34] or overestimating[26] dosage and dosing interval requirements. The enormous body size and dissimilar physiologic metabolism of elephants compared with other species, such as horses and cattle, complicate estimation of dosing requirements based on metabolic scaling calculations.[27,31,41]

Phenylbutazone, a pyrazolone NSAID, works primarily through inhibition of the arachidonic acid cascade to decrease production of prostaglandins and thromboxane. It is a potent pain reliever, antipyretic, and anti-inflammatory agent. Phenylbutazone has commonly been used in horses to treat bone and joint inflammation, laminitis, and soft-tissue inflammation[13] and in captive elephants has similarly been used to treat inflammatory conditions, such as arthritis.[31]

Potential negative side effects from use of phenylbutazone include gastrointestinal ulceration and hemorrhage, especially when used with other anti-inflammatory analgesics,[14,39,47] as well as inhibition of T_4 to T_3 conversion and decreased free thyroxine concentrations.[38] In one study with horses, chronic phenylbutazone administration affected articular cartilage metabolism, which might subsequently suppress proteoglycan synthesis and actually potentiate cartilage damage.[2] Other side effects may include kidney damage, suppression of white blood cell production, and aplastic anemia.[20,47]

Pharmacokinetic properties of phenylbutazone are known to vary significantly among different species,[32] and one author advises to ''avoid interspecies extrapolation of pharmacokinetic data . . . to extrapolate from one species to another is hazardous and increases the likelihood of adverse drug reactions.''[4] The maximum oral dose of phenylbutazone recommended by manufacturers for horses ranges from 1 to 3 mg/kg given t.i.d. Empirical dosages of phenylbutazone administered to captive elephants have ranged between 1 and 2 mg/kg every 24 hr[31]; however, in addition to problems with metabolic scaling, differences in NSAID dosage requirements between African and Asian elephants have been observed.[1]

For effective management of pain and inflammation in elephants, administration of phenylbutazone should occur when drug absorption is maxi-

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Table 1. Demographic characteristics of 10 African and 8 Asian elephant participants.

Species	Gender	No.	Weight (kg)	Age (yr)	Facility	Single-dose trials (mg/kg)	Multiple-dose trials
African	♂	Af Ma 1	7,000	23	Bowmanville Zoo[a]	2, 3, and 4	Completed
	♂	Af Ma 2	3,091	18	Riddle's Elephant Sanctuary[b]	2, 3, and 4	Completed
	♂	Af Ma 3	3,091	21	Riddle's Elephant Sanctuary	2, 3, and 4	Completed
	♂	Af Ma 4	4,091	22	Riddle's Elephant Sanctuary	2, 3, and 4	Completed
	♂	Af Ma 5	3,268	21	Wild Things, Inc.[c]	3 and 4	Completed
	♀	Af Fe 1	4,318	25	Kansas City Zoo[d]	2, 3, and 4	Completed
	♀	Af Fe 2	3,636	25	Kansas City Zoo	2, 3, and 4	Completed
	♀	Af Fe 3	3,600	25	Bowmanville Zoo	2, 3, and 4	Completed
	♀	Af Fe 4	4,064	24	Seneca Park Zoo[e]	2, 3, and 4	Completed
	♀	Af Fe 5	3,218	22	Wild Things, Inc.	3 and 4	Completed
Asian	♂	As Ma 1	6,136	42	Oregon Zoo[f]	2, 3, and 4	Completed
	♂	As Ma 2	3,400	15	Bowmanville Zoo	2, 3, and 4	Completed
	♂	As Ma 3	4,180	33	Bowmanville Zoo	Not done	Completed
	♂	As Ma 4	4,091	14	Riddle's Elephant Sanctuary	2, 3, and 4	Completed
	♀	As Fe 1	3,709	22	Oregon Zoo	2, 3, and 4	Not done
	♀	As Fe 2	3,200	40	Bowmanville Zoo	2, 3, and 4	Completed
	♀	As Fe 3	3,636	43	Riddle's Elephant Sanctuary	2, 3, and 4	Completed
	♀	As Fe 4	3,500	29	Riddle's Elephant Sanctuary	2, 3, and 4	Completed

[a] Bowmanville, Ontario, Canada.
[b] Greenbrier, Arkansas.
[c] Salinas, California.
[d] Kansas City, Missouri.
[e] Rochester, New York.
[f] Portland, Oregon.

mal and at a frequency that minimizes fluctuations in drug concentrations through time as for other animals.[49] The objective of this study was to determine appropriate dosing regimens for both African and Asian elephants based on noncompartmental pharmacokinetic analyses of phenylbutazone administered in both single and multiple doses.

MATERIALS AND METHODS

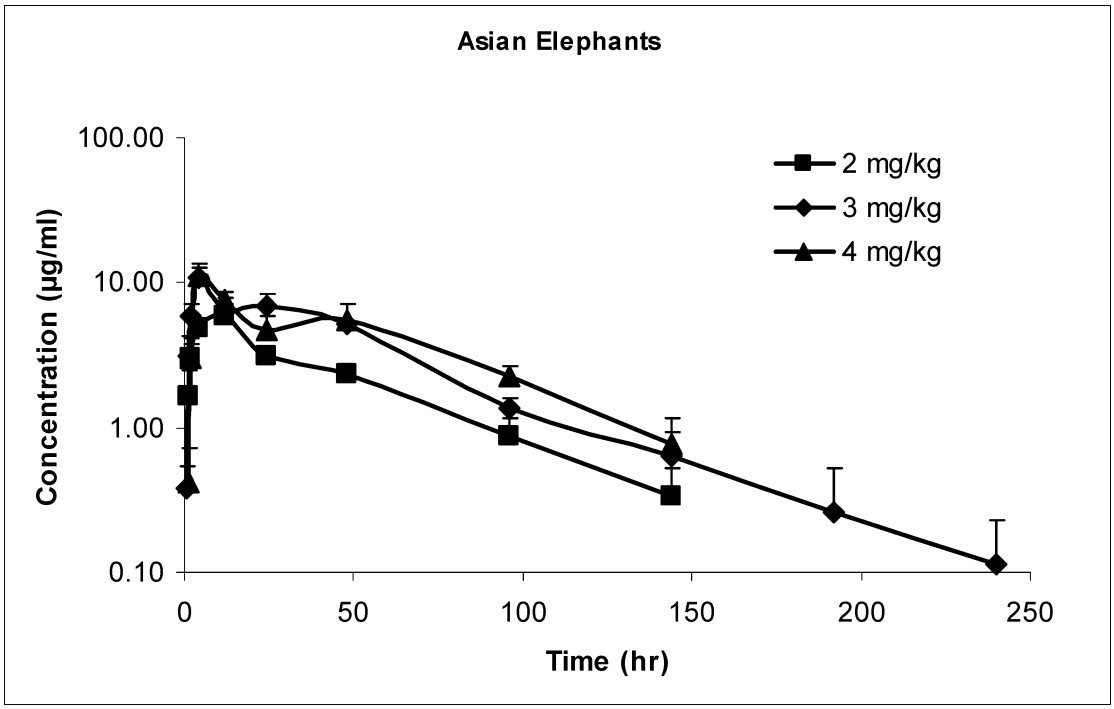
Animals

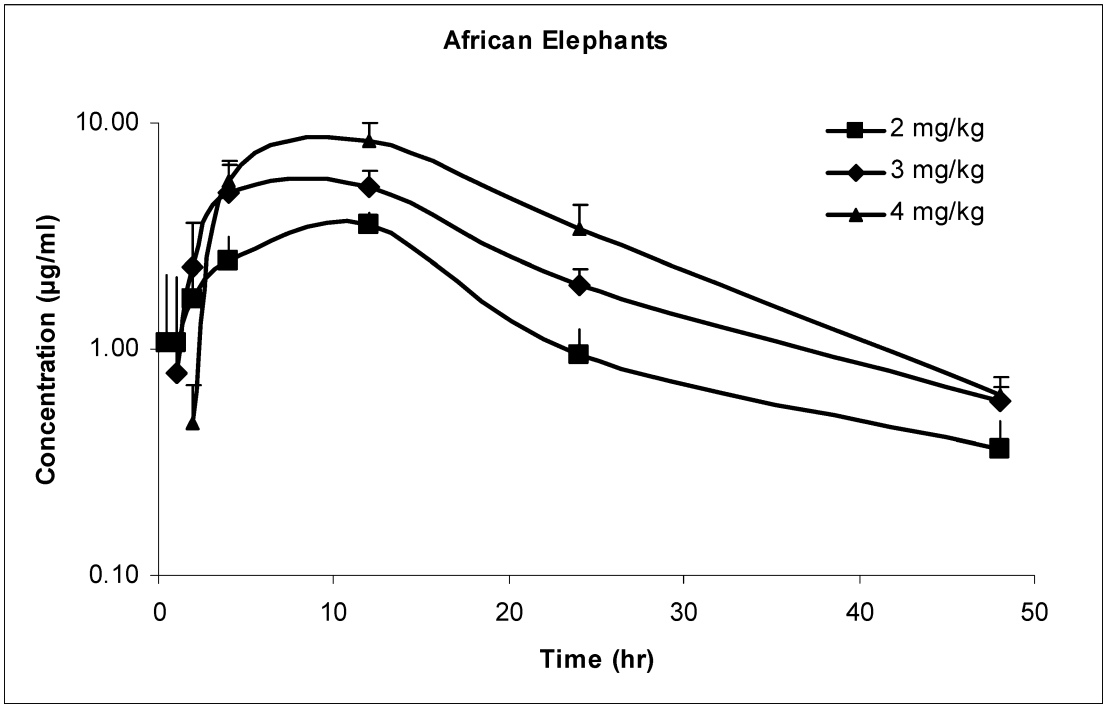
Eighteen healthy captive elephants (five male and five female African; four male and four female Asian) were used in this study (Table 1). Animals ranged in age from 14 to 43 yr and in weight from 3,218 to 4,318 kg for African and 3,200 to 6,136 kg for Asian elephants, and all animals were maintained in captivity in six facilities located in Canada and the USA. Phenylbutazone (Sigma Chemical Company, St. Louis, Missouri 63178, USA) was given orally with food treats (e.g., apples, bread, peanut butter), and blood samples were collected from ear veins using 20-gauge butterfly catheters.

Study design

Pilot studies were conducted using empirically derived dosing regimens to ensure that proper ranges for dosage and dosing frequency determinations would be utilized. Based on these results, single-dose trials using 2-, 3-, and 4-mg/kg dosages were initiated. Washout periods between trials were 4 wk in duration to allow for complete elimination of residual drug metabolites. Blood samples were collected at −5, 30, and 60 min; 2, 12, and 24 hr; and 2, 4, 6, 8, 10, and 12 days post administration. The −5 min samples were negative for detectable phenylbutazone after the 4-wk washout periods. Blood samples were placed into glass tubes and centrifuged for 10 min at 13,000 *g*. Serum was decanted into plastic screw-cap vials and kept frozen at −20°C. until time of analysis.

The optimal dosing frequency was determined by extrapolating serum concentrations of phenylbutazone from single-dose trials to steady-state levels. Based on these results, multiple-dose trials were initiated and consisted of 2-mg/kg doses given every 24 hr to African elephants and 3-mg/kg doses given every 48 hr to Asian elephants. Blood samples were collected hourly for 4 hr after each of three administrations and then every 6 hr plus 1 hr prior to the next administration for the 2-mg/kg dose. For the 3-mg/kg dose, blood samples were collected 3, 4, 12, 16, 28, 35, and 45 hr post administration plus 1 hr prior to the next administration for each of three administrations.





Phenylbutazone analysis

Serum phenylbutazone concentrations were quantified by high-performance liquid chromatography (HPLC) after sample preparation. To prepare samples, 0.5 ml of serum was added to 100 μl of internal standard (25 μg/mL of gemfibrozil), 1 ml of phosphate buffer (pH 7.4), and 1.5 ml of filtered water. The mixture was then vortexed for 30 sec and passed through a C_{18} solid phase extraction column that had been prewashed with 1 ml methanol and 1 ml filtered water. The residue was washed with 1.5 ml phosphate buffer (pH 7.4), 1.5 ml filtered water at 20 p.s.i., and then 0.15 ml acetonitrile. The column was vacuum dried, and the residue was eluted twice with 1.5 ml methanol, combined, and then vacuum dried again.

The samples were reconstituted with 0.5 ml mobile phase (40% acetonitrile, 60% 0.1 M acetic acid; pH 3.5) and vortexed for 1 min, followed by centrifugation for 5 min at 3,400 *g*. The reconstituted solution (100 μl) was injected onto an HPLC C-18 column at a flow rate of 1.5 ml/min. The HPLC system consisted of a pump (Model M-600 A, Water Associates, Inc., Milford, Massachusetts 01757, USA), an auto injector (Model 712, Water Associates, Inc.), a variable wavelength detector set at 229 nm (Model SP8773XR, Spectra Physics, San Jose, California 95154, USA), and an automated data integrator (Hewlett Packard Model 3390A, San Jose, California 95014, USA).

Serum concentrations of phenylbutazone were calculated from known standard concentrations (0.25–50 μg/ml), and a correlation coefficient for calibration curves was calculated ($r^2 = 0.98$, range 0.96–0.999). Assay sensitivity was 0.1 μg/ml with a 12.8% intra-assay coefficient of variation. The inter-assay coefficient of variation was 11.69%. Concentrations of oxyphenbutazone and γ-hydroxyphenylbutazone, active metabolites of phenylbutazone, were observed, but often sample concentration values were too low for accurate determination.

Pharmacokinetic and statistical analyses

Pharmacokinetic data were modeled and fitted using the software program Win NONLIN (2002 Version 3.2, PharSight, Mountain View, California 94040, USA) using noncompartmental analysis. Parameters determined for each elephant included the maximal concentration (C_{max}), time of maximal concentration (T_{max}), volume of distribution (V_d/F), area under the curve (AUC), mean residence time (MRT), clearance rate (Cl/F), terminal half-life ($T_{1/2}$), and the elimination rate constant (K_{el}). The elimination half-life for phenylbutazone ($T_{1/2,el}$) was determined by dividing the natural logarithm of 2 (0.693) by K_{el}.

Pharmacokinetic parameters were calculated for each elephant and each group of elephants based on species and gender (mean ± SD). The correlation between body weight and peak phenylbutazone concentrations as well as age and $T_{1/2}$ was analyzed by standard regression analysis (Excel, Microsoft, Redmond, Washington 98052, USA). Statistical comparisons between Asian and African elephants were done using an analysis of variance mixed effects model:

$$\log(y_{ijkl}) = s_i + z_j + \theta_k + \gamma_l + \varepsilon_{ijkl}$$

where y_{ijkl} represents the measured pharmacokinetic parameter on the *k*th dose in the *j*th zoo for the *i*th elephant of *l*th species. The random elephant effect is s_i, z_j is a fixed zoo effect, θ_k is the effect of the *k*th dose, γ_l is the effect of species, and ϵ_{ijkl} is the normally distributed random error with mean value zero. The parameter for zoo was included in the model to account for potential variability introduced by six geographically disparate zoos or private facilities. Statistical software was used for analysis (SAS, Version 8.0, InnaPhase Corporation, Cary, North Carolina 27513, USA).

Steady-state, single-dose linear kinetic analyses consisted of paired *t*-tests to compare Asian and African elephant AUC and clearance values. Estimates to predict appropriate doses and dosing intervals for phenylbutazone were based on pharmacokinetic results and literature values of identified therapeutic serum concentrations.

RESULTS

Serum concentrations of phenylbutazone versus time after administration of single doses of 2, 3, and 4 mg/kg are presented in Figure 1 (samples for 2- and 4-mg/kg doses in Asian elephants were not collected beyond 150 hr). In Asian elephants, a second peak in serum concentrations of phenylbutazone was observed in every elephant at every dose

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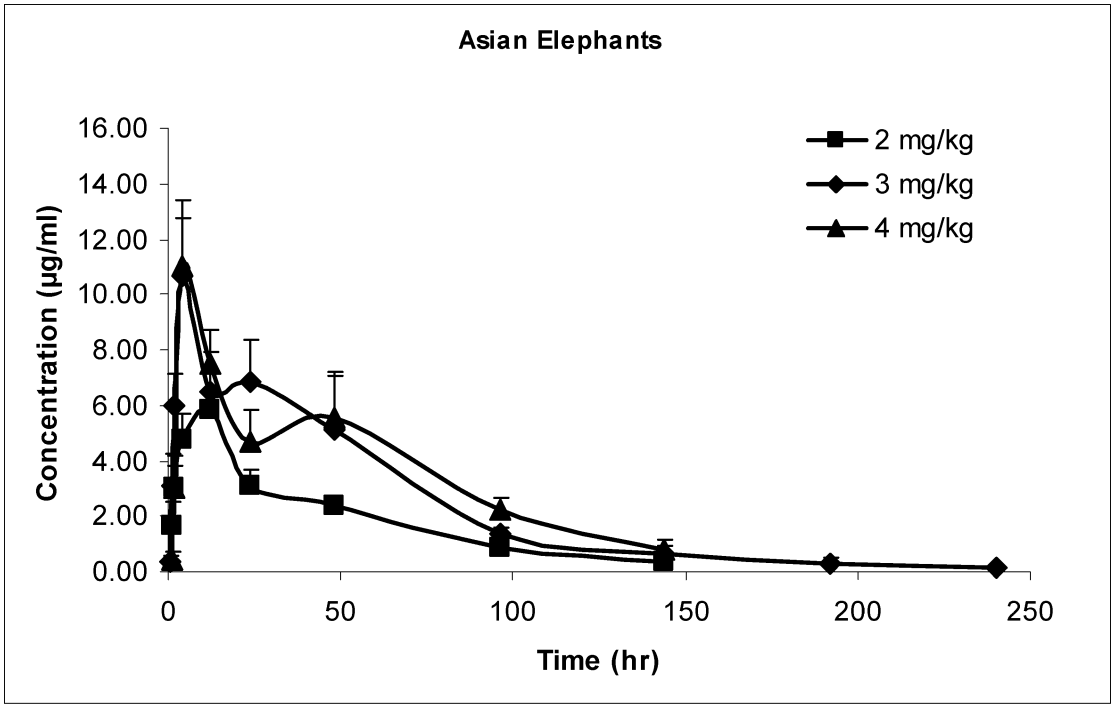
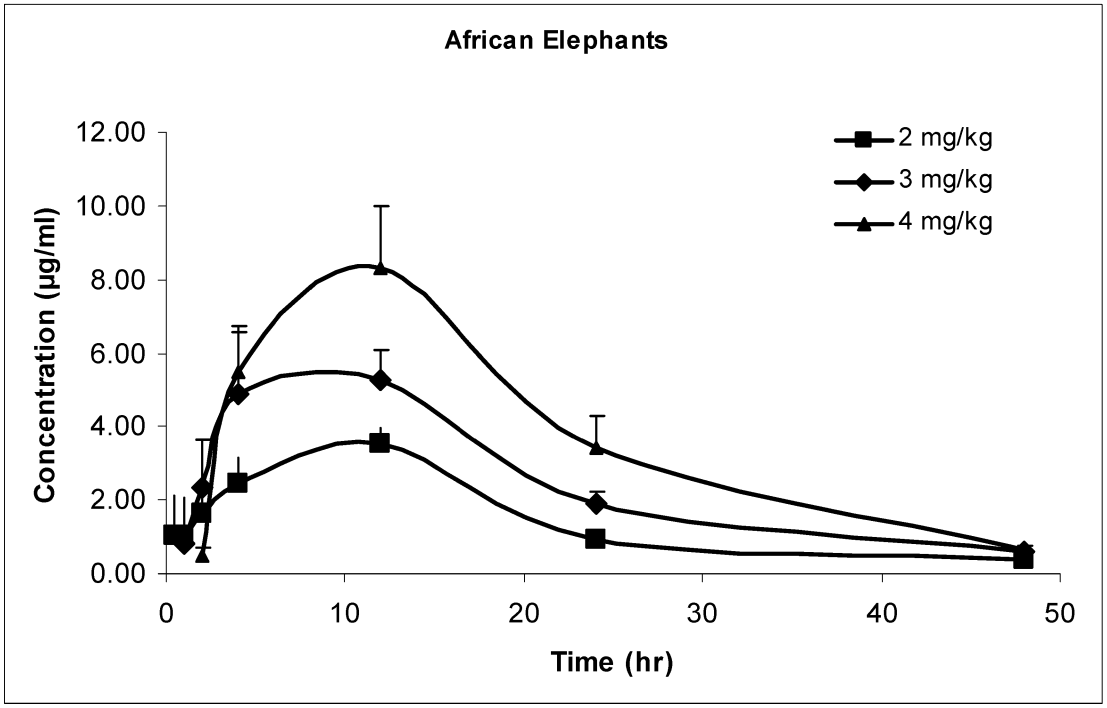
Figure 1. Serum concentrations of phenylbutazone (mean ± SD) in African and Asian elephants after oral administration of 2-, 3-, and 4-mg/kg dosages versus time on semilogarithmic plots to demonstrate elimination by linear kinetics.





given. Cartesian plots (Fig. 2) better illustrate phenylbutazone concentration versus time profiles for 2-, 3-, and 4-mg/kg single oral doses compared with semi-log or logarithmic plots and reveal the second phenylbutazone concentration peak in Asian elephants (especially for the 4-mg/kg dose). A second peak in serum concentrations of phenylbutazone was not seen in African elephants.

Serum concentrations of active phenylbutazone metabolites, oxyphenbutazone, and γ-hydroxyphenylbutazone were much lower than phenylbutazone concentrations in both Asian and African elephants. In African elephants, serum concentrations of oxyphenbutazone mirrored concentrations of phenylbutazone but at much lower levels, with peak concentrations at only 30%. Peak γ-hydroxyphenylbutazone concentrations reached only 8% of peak phenylbutazone concentrations in African elephants (1 μg/ml), but at most time points this metabolite was very difficult to quantify or was nonexistent. In Asian elephants, serum concentrations of oxyphenbutazone were lower compared with African elephants and were undetectable after 48 hr. Serum concentrations of γ-hydroxyphenylbutazone in Asian elephants were generally not quantifiable; however, small chromatographic peaks were present for the first 48 hr.

Pharmacokinetic differences in phenylbutazone metabolism between Asian and African elephants given a single oral dose of 2, 3, and 4 mg/kg are illustrated in serum concentration time profiles (Fig. 3), and pharmacokinetic parameters for phenylbutazone in Asian and African elephants are summarized in Table 2. Results for 2-mg/kg dosages for two African elephants at Wild Things, Inc., were not included because no or very little drug could be quantified in the serum, most likely due to difficulties encountered during administration (Table 1). There were significant differences in pharmacokinetic values between African and Asian elephants for AUC ($P < 0.0001$), Cl/F ($P < 0.0003$), $T_{1/2}$ ($P < 0.0001$), C_{max} ($P < 0.0071$), V_d ($P < 0.0138$), and MRT ($P < 0.0001$). The AUC increased progressively with increasing doses for each species, but the average range for African elephants was 68–171 μg/hr/ml versus 261–489 μg/hr/ml for Asian elephants. The V_d was slightly higher in African elephants (mean range: 590–605 ml/kg) compared with Asian elephants (mean range: 423–477 ml/kg). Average values for Cl and $T_{1/2}$ were also quite disparate between the two species (e.g., Cl of approximately 28-ml/hr/kg in African elephants versus 8-ml/hr/kg in Asian elephants).

Serum concentrations of phenylbutazone versus time profiles for Asian and African elephant multiple-dose trials are depicted in Figure 4. Asian elephants were given 3 mg/kg every 48 hr, and African elephants received 2 mg/kg every 24 hr. Pharmacokinetic parameters for phenylbutazone during multiple-dose trials are summarized in Table 3. Values do not include the female Asian elephant from the Oregon Zoo and do include an additional male Asian elephant from the Bowmanville Zoo (AsMa3 in Table 1) based on animal availability.

Serum concentrations of phenylbutazone between female and male elephants at each time point were nearly identical, and concentrations versus time graphs were similar. In this study, pharmacokinetic parameters for male and female African and Asian elephants were not statistically significant ($P > 0.5$ for each dose given).

A regression analysis was performed to see if pharmacokinetic parameters correlated with age and body weight. There was no statistical correlation with weight ($P > 0.15$), but there was a correlation between age and pharmacokinetic parameters for both Asian and African elephants ($P < 0.5$).

DISCUSSION

Phenylbutazone showed linear pharmacokinetics in both African and Asian elephants; however, the differences in parameters between species were remarkable. Different rates of absorption and drug metabolism attest to evolutionary-based divergence of physiologic systems between these two elephant species.

Absorption of phenylbutazone has been shown to vary considerably, especially when access to feed (such as hay) was provided, which delayed time to peak concentrations in horses by 6 to 12 hr[28,45] or more.[46] This also appears to be true in African and Asian elephants with peak serum concentrations of phenylbutazone occurring anytime from 4 to 24 hr post administration. Although this could not be confirmed, access to food (and potentially type of food) around time of administration appears to affect absorption of phenylbutazone in elephants.

The fraction of phenylbutazone bound to plasma

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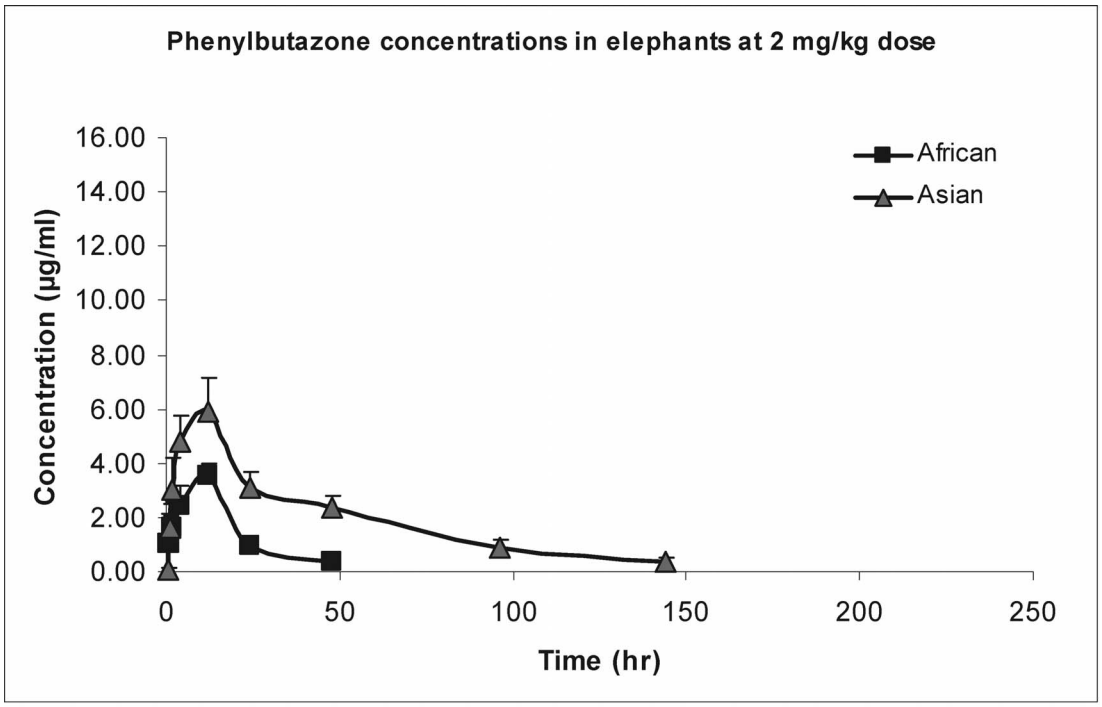
Figure 2. Cartesian plots to illustrate second peak in serum concentration-time profiles of phenylbutazone in Asian elephants.



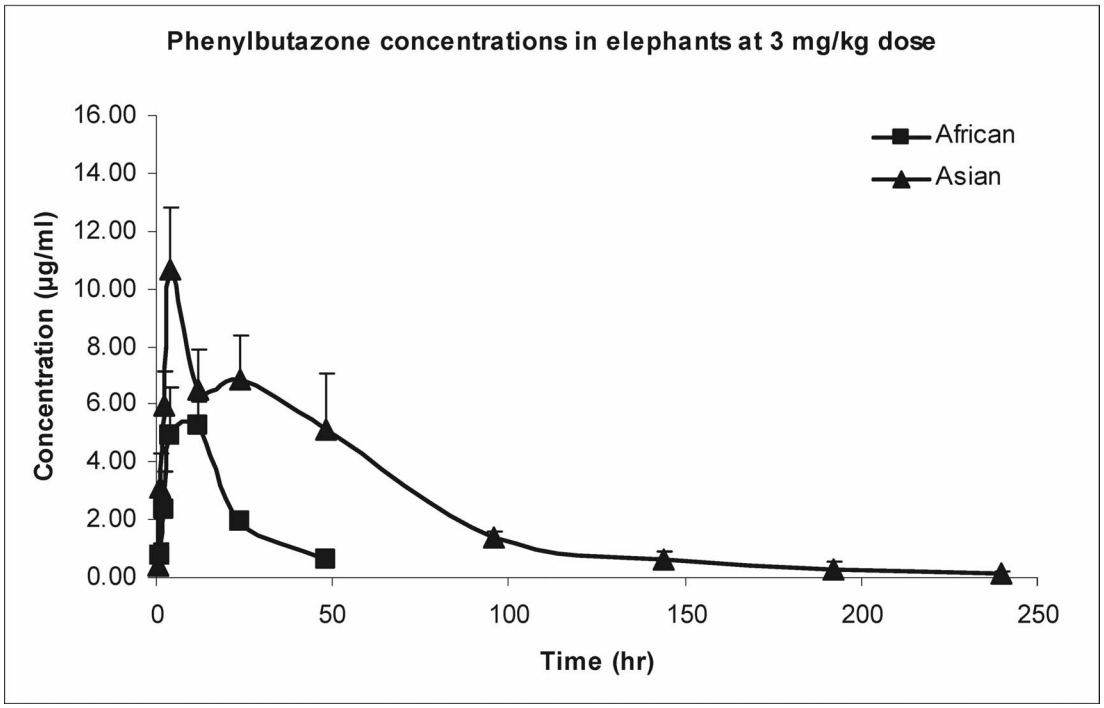


Figure 3. Concentration-time profiles of phenylbutazone after oral administration of **(a)** 2-, **(b)** 3-, and **(c)** 4-mg/kg dosages in African compared with Asian elephants.

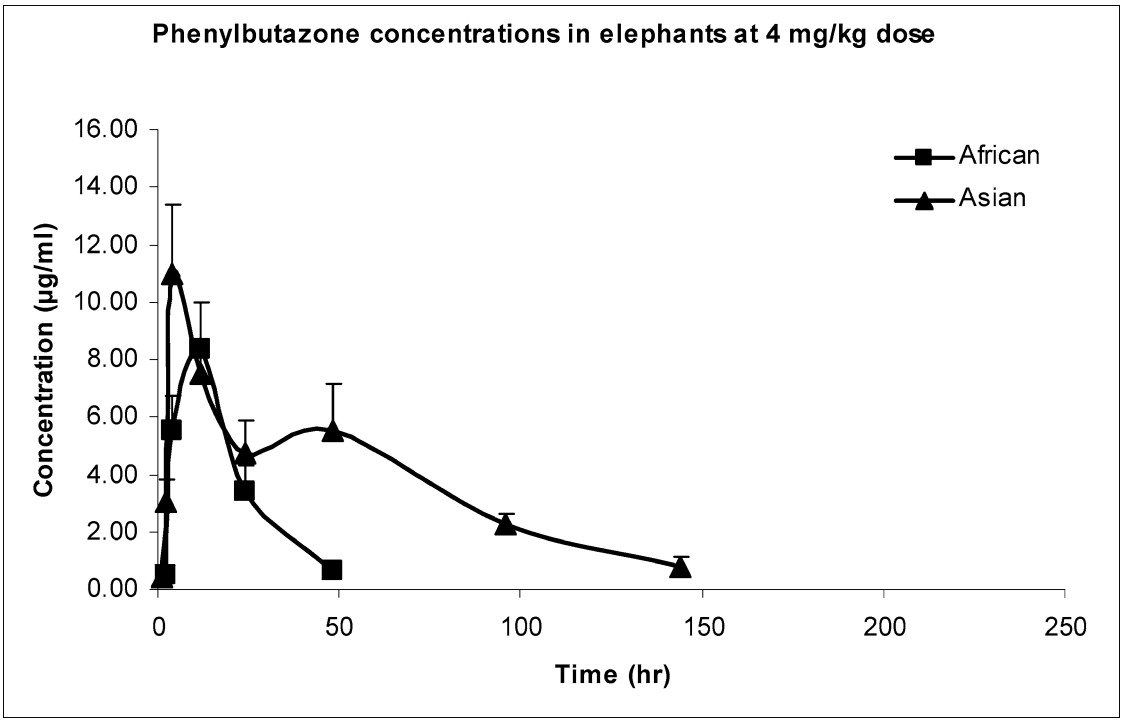


Figure 3. Continued.

proteins is 98–99%, which is similar across all species studied to date[32] and represents NSAIDs' normally highly protein-bound nature.[20] Protein binding decreases with increasing concentrations of drug.[5] A study conducted with lactating diary cows suggests that phenylbutazone either binds to milk proteins or is actively transported into milk because its concentration in milk was greater than that expected with simple partitioning from plasma into milk.[7] It is not known if phenylbutazone similarly binds to milk proteins in elephants, but females with nursing calves should probably not receive this drug.

Phenylbutazone follows zero-order (dose-dependent) elimination kinetics in dogs and horses and typically has a longer $T_{1/2,el}$ as the dose increases. The $T_{1/2,el}$ for phenylbutazone in cattle ranges from 35.9 hr[21] to 62.6 hr[49] but is much less in horses, ranging from 3.5 hr[36] to 5.5 hr.[24] Hepatic biotransformation results in secondary metabolites (mainly oxyphenbutazone and γ-hydroxy-phenylbutazone), and it has been suggested that elimination of phenylbutazone is inhibited by oxyphenbutazone.[19,35] This could contribute to the relatively long $T_{1/2}$ observed in elephants (14–45 hr). The plasma $T_{1/2}$ in horses has also been shown to increase as drug dosage levels increase, and this may indicate that the kinetics of phenylbutazone are dose dependent.[46] Phenylbutazone is primarily metabolized in the liver, and this process (hepatic mixed function oxidase activity) can become saturated, leading to decreased metabolic rates.[46,47] Enterohepatic cycling occurs by biliary excretion and intestinal reabsorption of solutes and is typically associated with multiple peaks and a longer apparent half-life in concentration time profiles.[40] It is through this process that higher dosages are likely to be associated with greater serum concentrations of drug over prolonged periods.

Enterohepatic cycling has been observed for a variety of compounds in numerous species (e.g., NSAIDs flurbiprofen[9] and ibuprofen[8] in rats and piroxicam in rabbits,[52] hormones estrone in bulls[25] and humans,[48] testosterone in dogs[29]). However, enterohepatic cycling of phenylbutazone is species specific, and some animals (e.g., llamas,[32] pigs[17]) do not exhibit evidence of cycling.

Asian, but not African, elephants experienced a second peak in serum concentrations of phenylbutazone, suggesting that enterohepatic cycling is occurring. Because the second peaks occurred during different times in individuals, mean concentration time curves do not fully and clearly show the extent of this. The multiple dose concentration versus time

Table 2. Pharmacokinetic parameters of phenylbutazone after oral administration of 2-, 3-, and 4-mg/kg doses in African and Asian elephants (mean ± SD).

Pharmacokinetic parameter	2 mg/kg		3 mg/kg		4 mg/kg		ANOVA[a]
	African (n = 8)	Asian (n = 7)	African (n = 10)	Asian (n = 7)	African (n = 10)	Asian (n = 7)	P value[b]
C_{max} (μg/ml)	4.26 ± 2.02	7.14 ± 2.36	7.22 ± 4.06	12.08 ± 3.13	9.72 ± 3.62	12.30 ± 5.01	0.0071
T_{max} (hr)	7.56 ± 4.88	9.71 ± 3.90	8.44 ± 4.42	14.00 ± 9.39	9.33 ± 4.00	12.67 ± 17.60	0.243
T½ (hr)[b]	15.05 ± 7.88	38.74 ± 24.27	13.3 ± 3.02	44.64 ± 21.55	13.57 ± 6.61	41.42 ± 15.68	0.0001
V_d/F (ml/kg)	605.8 ± 319.49	423.3 ± 172.24	590.6 ± 235.49	460.3 ± 89.51	591.0 ± 253.21	477.6 ± 198.55	0.0138
Cl/F (ml/hr/kg)[c]	27.98 ± 15.69	7.63 ± 3.20	26.86 ± 8.76	7.77 ± 3.10	28.26 ± 14.46	8.21 ± 3.29	0.0003
AUC (μg/hr/ml)	68.0 ± 29.56	261.1 ± 137.25	116.0 ± 47.84	387.4 ± 17.93	171.2 ± 96.86	489.0 ± 236.42	0.0001
MRT (hr)	22.52 ± 10.89	55.51 ± 27.61	21.85 ± 3.66	54.48 ± 19.45	22.13 ± 4.95	56.86 ± 19.87	0.0001

[a] ANOVA, analysis of variance; C_{max}, maximal concentration; T_{max}, time of maximal concentration; T½, terminal half-life; V_d/F, volume of distribution; Cl/F, clearance rate; AUC, area under the curve; MRT, mean residence time.

[b] P value represents the statistical difference between African and Asian elephants.

[c] Harmonic means.

curves (Fig. 4) for Asian elephants best depict these second concentration peaks, and occasionally a third peak was even observed. Third peaks have also been observed during enterohepatic cycling of estrone in women,[48] in which C_{max} of the second absorption of estrone was 20% that of the first and for the third circulation was half that of the second. Because enterohepatic cycling of phenylbutazone is likely occurring in Asian elephants, the $T_{1/2}$ presented in Table 2 for Asian elephants is an apparent half-life for the drug.

Enterohepatic cycling may be more pronounced in animals with prolonged gastrointestinal residence times[37] or longer intestinal tracts, which could provide greater surface area for absorption. Asian elephants have higher digestion coefficients and longer ingesta mean retention times than African elephants on comparable diets.[12,15] A recent study supports earlier work to demonstrate that small intestinal tracts in Asian elephants are longer,[6] ranging from 15.9 m[43] to 22.6 m[11] compared with African elephants, which range from 9.2 m[6] to 13.8 m.[44] This difference in length between elephant species occurs in both the small and large intestines and is postulated to be based on differences in natural diet, with Asian elephants' diet composed of a higher proportion of grass. In this study, these anatomical differences might also account for enterohepatic cycling seen with phenylbutazone only in Asian, not African, elephants.

It is prudent to also consider age, gender, and health status when calculating dosage requirements for elephants. It has been shown that there are significant differences in drug elimination between males and females in several mammalian species,[23,51] including humans,[50] and there is no reason to believe that elephants are different in this respect. A recent study conducted with African and Asian elephants suggested that slightly lower dosages be used in females than males, although larger sample sizes would be needed to demonstrate statistical significance.[1] Even though differences in pharmacokinetic parameters with respect to gender were not demonstrated in this study, results do not preclude this possibility.

Physiologic state can also affect the pharmacokinetic parameters of drugs and recommendations for their use. Statistical differences in the Cl, V_d, and $T_{1/2}$ of penicillin-G were found between pregnant and lactating sheep,[3] and phenylbutazone can be toxic to the embryos of mares.[47] Certainly illnesses adversely affecting drug metabolism can have profound impacts on the pharmacokinetic disposition of phenylbutazone and possibly exacerbate existing conditions in the liver and kidneys. In one

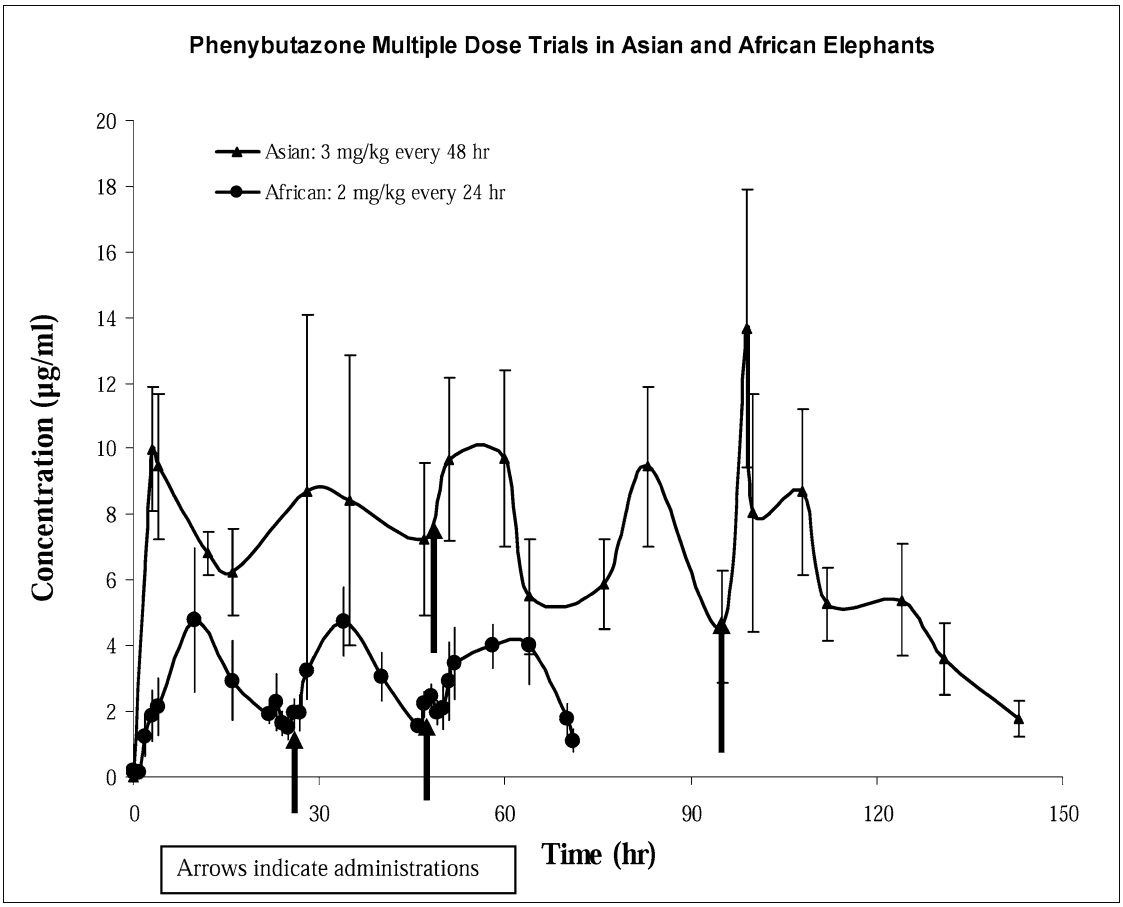


Figure 4. Concentration-time profiles of phenylbutazone after oral administration of multiple 2- and 3-mg/kg dosages in African and Asian elephants, respectively (arrows represent time of administrations).

study, accelerated elimination of phenylbutazone in uremic patients was thought to be caused by altered drug distribution related to decreased serum protein binding.[16] Consideration of an elephant's general health status, especially with respect to the gastrointestinal system and organs of elimination, as well as normal physiologic state (e.g., pregnancy, musth), should be given prior to administering phenylbutazone. Additionally, concomitant administration of other drugs, especially other NSAIDS and anticoagulants, should be noted because phenylbutazone can amplify the effects of these drugs[39,47] presumably through competitive plasma protein binding.

Effects of age on phenylbutazone pharmacokinetics have been observed in several species, including goats.[10] Clearance of phenylbutazone in horses was found to be age dependent, with decreased Cl in older individuals,[22] and the $T_{1/2,el}$ for phenylbutazone in cattle varies among age groups as well.[21,42,49] Similar findings were observed in a previous study with elephants with respect to Cl of ibuprofen and age,[1] and a correlation between age and phenylbutazone $T_{1/2}$ was also demonstrated in this study for both Asian and African elephants ($P < 0.5$). These results suggest that the dose of phenylbutazone in elderly elephants should be reduced.

The minimum effective concentration for phenylbutazone is not known for any species,[32] but for horses a target range of 5–15 µg/ml has been suggested.[22] In this study, average steady state serum concentrations of phenylbutazone in Asian elephants given 3-mg/kg doses every 48 hr were 6.9 ± 2.49 µg/ml. The response to phenylbutazone treatment for chronic musculoskeletal pain suggests that this 3-mg/kg dose for Asian elephants is effective. A 32-yr-old female Asian elephant at the Rio Grande Zoo in Albuquerque, New Mexico, was treated with phenylbutazone to manage left hind limb lameness (Richard, pers. comm.). She was

Table 3. Steady-state pharmacokinetic parameters (mean ± SD) of phenylbutazone after oral multiple dosing in African elephants (2 mg/kg dose every 24 hr) and Asian elephants (3 mg/kg dose every 48 hr).

Parameter	African elephants (2 mg/kg q 24 hr; n = 10)	Asian elephants (3 mg/kg q 48 hr; n = 7)
C_{max} (μg/ml)[a]	5.4 ± 2.67	15.6 ± 10.40
C_{min} (μg/ml)	1.1 ± 0.39	1.2 ± 1.36
C_{avg} (μg/ml)	3.0 ± 1.60	6.9 ± 2.49
T_{max} (hr)	11.8 ± 4.29	9.0 ± 6.06
Cl/F (ml/hr/kg)[b]	28.3 ± 36.25	9.1 ± 13.69
AUC (μg/hr/ml)	70.6 ± 36.56	330.0 ± 116.21
MRT (hr)	25.0 ± 13.37	55.8 ± 4.50
T½ (hr)[b]	13.4 ± 1.96	38.7 ± 2.81

[a] C_{max}, maximal concentration; C_{min}, minimal concentration; C_{avg}, average concentration; T_{max}, time of maximal concentration; Cl/F, clearance rate; AUC, area under the curve; MRT, mean residence time; T½, terminal half-life.

[b] Harmonic means.

given 1–2 mg/kg orally per day (2–4 mg/kg in 48 hr), which resulted in a noticeable improvement in mobility based on observations. Interestingly, this same dosage was administered rectally as a slurry with warm water on an every-other-day basis for 10 mo with similar results. Long-term maintenance dosing regimens of 1 mg/kg per day administered either orally or rectally resulted in no negative side effects. In a separate case study, a 19-yr-old female captive Asian elephant was treated for a severe sole abscess surgically, and then 1.7 mg/kg phenylbutazone was administered orally twice daily (3.4 mg/kg in 48 hr) for 1 wk with good results.[33] Phenylbutazone is eliminated more slowly in Asian elephants because of enterohepatic cycling; therefore, it is recommended that phenylbutazone be administered in 3-mg/kg doses given every 48 hr (instead of potentially lower dosages given more frequently as described above).

In comparison, African elephants require more frequent dosing because their Cl rate was comparatively higher and they did not exhibit enterohepatic cycling. The average steady-state serum concentrations of phenylbutazone in African elephants given 2-mg/kg doses every 24 hr were 3.0 ± 1.60 μg/ml. This is slightly below the target range of 5–15 μg/ml suggested as a minimum effective concentration for phenylbutazone in horses.[22] The AUC (associated with bioavailability) for respective dosing regimens in African elephants was 68 ± 29.6 μg/hr/ml compared with 387.4 ± 17.93 μg/hr/ml in Asian elephants. A slightly higher dose administered orally at 3 mg/kg every 24 hr might be appropriate for African elephants; however, long-term maintenance using the lower dosing regimen is being recommended. Bioavailability is affected by a variety of factors, including intestinal absorption and gut-wall metabolism,[40] and the V_d for African elephants given the 2-mg/kg dose was 605.8 ± 319.49 ml/kg, which compares with 460.3 ± 89.51 ml/kg in Asian elephants receiving the 3-mg/kg dose. This means that phenylbutazone is more broadly distributed across tissues in African than in Asian elephants. Also, at higher dosages, hepatic mixed function oxidase activity can become saturated and lead to decreased metabolic rates or longer elimination $T_{1/2}$.[47] Finally, because phenylbutazone is highly protein bound, a small reduction in binding (perhaps due to displacement by another drug) can have significant pharmalogic effects (e.g., assuming that phenylbutazone is 97% bound to albumin and there is just a 3% reduction in binding, the free drug concentration effectively doubles). Future long-term, multiple-dose trials using 3 mg/kg every 24 hr in African elephants could confirm which dosing regiment would be most appropriate.

CONCLUSIONS

The pharmacokinetic parameters of phenylbutazone differed significantly for C_{max}, $T_{1/2}$, V_d, Cl, AUC, and MRT between African and Asian elephants. Asian elephants exhibited enterohepatic cycling of phenylbutazone, which extended Cl rates significantly. Potentially therapeutic doses of phenylbutazone for Asian elephants appear to be 3 mg/kg given every 48 hr and 2 mg/kg given every 24 hr for African elephants. Larger sample sizes are required to confirm the differences observed relative to age. Gender, health status, and physiologic state should also be considered when calculating phenylbutazone dosage regimens for individual elephants. Although no side effects were seen during this study, trials exploring long-term administration of phenylbutazone were not conducted, so confirmation of the safety of phenylbutazone when used at these recommended dosage levels for treatment of chronic conditions could not be determined. Veterinarians are advised to maintain awareness of the potential for occasional upper gastrointestinal bleeding and blood dyscrasias and to monitor hepatic and renal function during the course of long-term phenylbutazone administration. Additionally, concurrent administration of other drugs should be evaluated for their possible effects on the metabolism and potentiating effects of phenylbutazone.

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