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

Authors: Bechert, Ursula, and Christensen, J. Mark

Source: Journal of Zoo and Wildlife Medicine, 38(2) : 258-268

Published By: American Association of Zoo Veterinarians

URL: https://doi.org/10.1638/1042-7260(2007)038[0258:POOAII]2.0.CO;2

Journal of Zoo and Wildlife Medicine 38(2): 258–268, 2007

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

Ursula Bechert, D.V.M., Ph.D. and J. Mark Christensen, Ph.D.

Abstract: The pharmacokinetic parameters of S(+) and R(−) ibuprofen were determined in 20 elephants after oral administration of preliminary 4-, 5-, and 6-mg/kg doses of racemic ibuprofen. Following administration of 4 mg/kg ibuprofen, serum concentrations of ibuprofen peaked at 5 hr at 3.9 ± 2.07 μg/ml R(−) and 10.65 ± 5.64 μg/ml S(+) (mean ± SD) in African elephants (*Loxodonta africana*) and at 3 hr at 5.14 ± 1.39 μg/ml R(−) and 13.77 ± 3.75 μg/ml S(+) in Asian elephants (*Elephas maximus*), respectively. Six-milligram/kilogram dosages resulted in peak serum concentrations of 5.91 ± 2.17 μg/ml R(−) and 14.82 ± 9.71 μg/ml S(+) in African elephants, and 5.72 ± 1.60 μg/ml R(−) and 18.32 ± 10.35 μg/ml S(+) in Asian elephants. Ibuprofen was eliminated with first-order kinetics characteristic of a single-compartment model with a half-life of 2.2–2.4 hr R(−) and 4.5–5.1 hr S(+) in African elephants and 2.4–2.9 hr R(−) and 5.9–7.7 hr S(+) in Asian elephants. Serum concentrations of R(−) ibuprofen were undetectable at 24 hr, whereas S(+) ibuprofen decreased to below 5 μg/ml 24 hr postadministration in all elephants. The volume of distribution was estimated to be between 322 and 356 ml/kg R(−) and 133 and 173 ml/kg S(+) in Asian elephants and 360–431 ml/kg R(−) and 179–207 ml/kg S(+) in African elephants. Steady-state serum concentrations of ibuprofen ranged from 2.2 to 10.5 μg/ml R(−) and 5.5 to 32.0 μg/ml S(+) (mean: 5.17 ± 0.7 R(−) and 13.95 ± 0.9 S(+) μg/ml in African elephants and 5.0 ± 1.09 μg/ml R(−) and 14.1 ± 2.8 μg/ml S(+) in Asian elephants). Racemic ibuprofen administered at 6 mg/kg/12 hr for Asian elephants and at 7 mg/kg/12 hr for African elephants results in therapeutic serum concentrations of this antiinflammatory agent.

Key words: African elephants, Asian elephants, ibuprofen, pharmacokinetics.

INTRODUCTION

Roughly 11,000 Asian elephants (*Elephas maximus*) live in logging camps, circuses, and zoos around the world, and approximately 250 African elephants (*Loxodonta africana*) live in captivity in North America alone. There is a high prevalence of musculoskeletal disorders (e.g., trauma, arthritis) among captive elephants, reported to occur in over 70% of the population in North America in a retrospective study.[15]

To treat these and other conditions, nonsteroidal antiinflammatory agents (NSAIDS), like ibuprofen, are used strictly on an empirical basis in elephants. Only one other pharmacokinetic study has been conducted with NSAIDS in elephants (ketoprofen);[7] but several studies have been published for various antibiotics.[3,4,13,18,20,21] Results from these studies suggest that metabolic scaling calculations for elephants are unreliable, either under-[18] or overestimating[13] dosage and dosing interval requirements. The enormous body size and dissimilar physiologic metabolism of elephants compared to other species, such as horses and cows, complicate estimation of dosing requirements based on metabolic scaling calculations.[16,22]

The elimination rate and half-life of a drug may be profoundly affected by several factors, including variations in metabolic rate between species. There is some indication that species differences in drug metabolism exist between African and Asian elephants, although this has not been substantiated.[16,22] Furthermore, variations in drug metabolism have been reported in certain species based on time of administration (i.e., diurnal variation)[26] and gender.[24] For effective management of pain and inflammation in elephants, administration of NSAIDS should occur when drug absorption is maximal and at a frequency that minimizes fluctuations in drug concentrations through time.

Ibuprofen is a propionic acid derivative with analgesic and antipyretic properties, and is found in high concentrations in synovial fluid, which is a proposed site of action.[23] The S(+) enantiomer is largely responsible for inhibition of prostaglandin synthesis by blocking cyclooxgenase activity. The R(−) enantiomer has minimal activity as an antiinflammatory agent and may be responsible for the undesirable effects of ibuprofen.[2] Empirical dosages of ibuprofen administered to captive elephants range between 0.5 and 4.0 mg/kg, and the median dosing frequency used by zoo veterinarians for this

From College of Science, Oregon State University, 2082 Cordley Hall, Corvallis, Oregon 97331, USA (Bechert); College of Pharmacy, Oregon State University, Corvallis, Oregon 97331, USA (Christensen). Correspondence should be directed to Dr. Bechert (ursula.bechert@oregonstate.edu).

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

Species	Gender	ID no.	Weight (kg)	Age (yr)	Facility
African	♂	Af Ma1	2318	18	Riddle's Elephant Sanctuary[a]
	♂	Af Ma2	4091	22	Riddle's Elephant Sanctuary
	♂	Af Ma3	3091	21	Riddle's Elephant Sanctuary
	♂	Af Ma4	3091	18	Riddle's Elephant Sanctuary
	♂	Af Ma5	6095	23	Bowmanville Zoo[b]
	♀	Af Fe1	3571	25	Bowmanville Zoo
	♀	Af Fe2	3364	24	Kansas City Zoo[c]
	♀	Af Fe3	3636	24	Kansas City Zoo
	♀	Af Fe4	3748	22	Pittsburgh Zoo[d]
	♀	Af Fe5	2727	18	Riddle's Elephant Sanctuary
Asian	♂	As Ma1	5205	40	Oregon Zoo[e]
	♂	As Ma2	6162	42	Oregon Zoo
	♂	As Ma3	4629	32	Bowmanville Zoo
	♂	As Ma4	4091	13	Riddle's Elephant Sanctuary
	♀	As Fe1	3500	29	Riddle's Elephant Sanctuary
	♀	As Fe2	3636	43	Riddle's Elephant Sanctuary
	♀	As Fe3	3171	40	Bowmanville Zoo
	♀	As Fe4	2455	36	Have Trunk Will Travel[f]
	♀	As Fe5	4000	33	Have Trunk Will Travel
	♀	As Fe6	3709	20	Oregon Zoo

[a] Greenbrier, Arkansas.
[b] Bowmanville, Ontario, Canada.
[c] Kansas City, Missouri.
[d] Pittsburgh, Pennsylvania.
[e] Portland, Oregon.
[f] Perris, California.

drug is 24 hr.[16] The average dose given to humans is 7 mg/kg, and therapeutic serum concentrations range between 15 and 30 mg/L.[26] Chronic use of ibuprofen can lead to impairment of hepatic or renal function,[26] or cause upper gastrointestinal tract bleeding,[3] necessitating determination of safe and therapeutic dosing regimens for elephants. The objectives of our research were 1) to determine the pharmacokinetic parameters of S(+) ibuprofen and R(−) ibuprofen after oral administration of racemic ibuprofen to elephants, 2) to compare values between elephant species as well as between males and females, and 3) to design therapeutic dosing regimens that maintain serum ibuprofen concentrations at or slightly above levels known to be efficacious in other species for treatment of musculoskeletal disorders and inflammatory conditions.

MATERIALS AND METHODS

Animals

Twenty elephants (five male and five female African; and four male and six female Asian) housed in zoos or private facilities throughout North America were used in this research (Table 1). Animals were deemed healthy based on results from complete blood counts, serum chemistry values, and general physical assessments as well as keeper observations conducted at the beginning of the study. Weights were obtained prior to initiation of the study and ranged between 3,400 and 4,400 kg for African and 3,600 and 4,900 kg for Asian elephants. Animals were 14 and 44 yr of age. Ibuprofen was given orally with food treats (e.g., apples, bread), and blood samples were taken from superficial ear veins with the use of 20-gauge butterfly catheters, which allowed for some animal movement during venipuncture. None of the elephants had received any medications for at least 4 wk prior to initiation of this research.

Study design

Pilot studies were conducted at the Oregon Zoo with the use of empirically derived dosing regimens and preceded each set of clinical trials to ensure that proper ranges for dosage and dosing frequency determinations would be utilized. The best therapeutic dose for racemic ibuprofen was determined by using 4-, 5-, and 6-mg/kg dosages in each animal. Washout periods between trials were 3 wk in duration and allowed for complete elimination of

Table 2. Pharmacokinetic parameters of R(−) and S(+) chiral ibuprofen after oral administration of 4-, 5-, 6-mg/kg racemic ibuprofen.

Pharmacokinetic parameter	African elephants (4 mg/kg)		Asian elephants (4 mg/kg)		African elephants (5 mg/kg)	
	R(−)	S(+)	R(−)	S(+)	R(−)	S(+)
C_{max} (μg/ml)	3.90 ± 2.07	10.65 ± 5.64	5.14 ± 1.39	13.77 ± 3.75	5.00 ± 2.90	12.39 ± 6.10
T_{max} (hr)	5.00 ± 2.71	5.00 ± 2.71	3.06 ± 1.32	3.06 ± 1.32	4.00 ± 0.00	4.00 ± 0.00
Half-life (hr) (harmonic mean)	2.27 ± 3.31	4.49 ± 3.81	2.40 ± 0.60	5.90 ± 1.83	2.43 ± 0.73	4.59 ± 1.93
V_d/F (ml/kg)	431.2 ± 94.0	185.0 ± 85.4	321.6 ± 83.6	133.7 ± 35.1	417.3 ± 83.6	178.5 ± 75.0
Cl/F (ml/hr/kg)	66.9 ± 16.5	21.6 ± 9.92	50.2 ± 9.80	14.1 ± 5.5	66.8 ± 26.5	21.6 ± 8.7
Cl/F (ml/hr/kg) (harmonic mean)	60.2 ± 16.3	20.4 ± 9.90	45.2 ± 9.70	13.3 ± 5.5	60.2 ± 26.0	20.3 ± 8.7
AUC (μg/hr/ml)	26.92 ± 14.8	88.51 ± 46.64	39.79 ± 12.58	141.64 ± 5.5	37.70 ± 14.02	115.9 ± 42.11
MRT (hr)	6.45 ± 3.41	9.19 ± 3.81	6.40 ± 0.90	9.47 ± 1.35	6.30 ± 2.02	8.30 ± 2.75
K_a (hr)	0.439 ± 0.410	0.464 ± 0.403	0.559 ± 0.238	0.436 ± 0.305	0.353 ± 0.381	0.404 ± 0.333

residual drug metabolites. Blood samples were collected at −5, 15, 30, 45, and 60 min; and 1.5, 2, 4, 10, 12, 24, and 48 hr postadministration from all elephants. Samples were placed into glass tubes and centrifuged for 10 min at 13,000 *g*. The serum was decanted into plastic screw-cap vials and kept frozen until time of analysis.

The optimal dosing frequency was determined by examining two different dosing intervals (12 and 24 hr) utilizing the therapeutic dosage. Simulations were performed to extrapolate ibuprofen serum concentrations to steady-state levels with the use of the single-dose ibuprofen concentrations. Based on these results, a 12-hr multiple dosing interval was selected. Blood samples were collected hourly for 4 hr after each of three administrations, then every 6 hr plus 1 hr prior to the next administration.

Ibuprofen analysis

Serum ibuprofen concentrations were quantified by high-performance liquid chromatography (HPLC) after sample preparation.[12,17] Briefly, 100 μl of internal standard solution (100 μg/ml gemfibrozil) and three drops glacial acetic acid (to denature protein) were added to each 0.5 ml of serum, and the solution was vortexed for 30 sec to ensure thorough mixing. To this solution, 0.5 ml acetonitrile was added, followed by vortexing for 30 sec. Ibuprofen was double extracted with the use of 2 ml of cyclohexane and vortexing for 30 sec followed by centrifugation for 10 min at 1,500 *g*. The supernatant was separated, vacuum evaporated, and dried. The dried samples were then reconstituted with 0.5 ml of mobile phase (0.01 M ammonium acetate in methanol) and vortexed for 1 min, followed by centrifugation for 10 min at 1,500 *g*. Of this solution, 100 μl was injected onto an HPLC C-18 (Chirex 5-μm chiral 3005 column; Phenomenex, Torrance, California 09051) column at a flow rate of 0.5 ml per 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., Milford, Massachusetts 01757, USA), 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 S(+) ibuprofen and R(−) ibuprofen were calculated from known standard concentrations (0.1, 0.5, 1, 5, 10, 25, and 50 μg/ml), and a correlation coefficient for calibration curves was calculated ($r^2 = 0.998$). Assay sensitivity was 0.1 μg/ml with a 12.9% intraassay coefficient of variation. The interassay coefficient of variation was 6.4%.

Pharmacokinetic and statistical analyses

Pharmacokinetic data for each enantiomer of ibuprofen were modeled and fitted with the use of the software program Win NONLIN (2002 Version 3.2; PharSight, Mountain View, California 94040, USA) by noncompartmental analysis. Parameters determined for each enantiomer included the absorption rate constant (K_a), 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 ibuprofen ($T_{1/2el}$) was determined by dividing the natural logarithm of 2/0.693 by K_{el}.

Pharmacokinetic parameters for each ibuprofen

Table 2. Extended.

Asian elephants (5 mg/kg)		African elephants (6 mg/kg)		Asian elephants (6 mg/kg)	
R(−)	S(+)	R(−)	S(+)	R(−)	S(+)
5.40 ± 2.54	13.97 ± 6.59	5.91 ± 2.17	14.82 ± 9.71	5.72 ± 1.60	18.32 ± 10.35
4.17 ± 2.40	4.17 ± 2.40	6.44 ± 3.43	6.44 ± 3.43	4.67 ± 3.84	4.67 ± 3.84
2.60 ± 0.36	6.37 ± 1.04	22.28 ± 0.88	5.10 ± 4.59	2.93 ± 0.59	7.67 ± 3.00
356.5 ± 53.4	157.6 ± 50.0	359.7 ± 94.9	206.5 ± 101.4	356.6 ± 58.6	172.7 ± 62.2
59.5 ± 22.5	14.7 ± 5.7	66.4 ± 15.4	20.1 ± 9.4	62.5 ± 5.60	13.8 ± 5.6
53.4 ± 22.4	13.9 ± 5.6	59.8 ± 15.1	18.9 ± 9.2	56.3 ± 5.8	13.0 ± 5.7
42.06 ± 16.83	170.2 ± 68.00	45.27 ± 21.68	149.1 ± 72.88	48.33 ± 12.18	218.22 ± 110.2
6.00 ± 0.90	10.75 ± 1.70	5.97 ± 1.73	10.27 ± 3.06	5.75 ± 2.36	12.55 ± 5.17
0.364 ± 0.217	0.365 ± 0.215	0.441 ± 0.435	0.409 ± 0.403	0.505 ± 0.238	0.637 ± 0.305

enantiomer were calculated for each elephant and each group of elephants based on species and gender (mean ± SD). The correlation between body weight and peak R(−) and S(+) ibuprofen concentrations was analyzed by standard regression analysis (Excel; Microsoft, Redmond, Washington 98052, USA). Statistical comparisons between Asian and African elephants were done with the use of an ANOVA 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 kth dose in the jth zoo for the ith elephant of lth species. The random elephant effect is s_i, z_j is a fixed zoo effect, θ_k is the effect of the kth 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 for each enantiomer. Estimates to predict appropriate doses and dosing intervals for ibuprofen were based on literature values of identified therapeutic serum concentrations.

RESULTS

Serum concentrations of R(−) and S(+) ibuprofen at various times after p.o. administration of different dosages are plotted in Figure 1a. Initial serum concentrations of ibuprofen revealed that concentrations of the two isomers of ibuprofen, R(−) and S(+), were in the body at equal concentrations. Over time in both Asian and African elephants, the R(−) isomer of ibuprofen decreased faster than the S(+) isomer until at 24 hr, no detectable R(−) isomer of ibuprofen remained; only the S(+) isomer was present. Initial serum concentrations of the R(−) and S(+) ibuprofen enantiomers were equal during the absorption phase, but the concentrations of R(−) to S(+) isomer were lower in all female elephants. Little variation was seen among female elephants in their pattern of elimination of R(−) and S(+) ibuprofen over time. However, male elephants showed a greater variation in the elimination of R(−) ibuprofen in plasma compared to S(+) ibuprofen. Younger male Asian elephants (less than 33 years of age) experienced a more rapid and extensive decrease in plasma concentrations of R(−) compared to S(+) than older Asian elephants. Younger males had S(+) levels of ibuprofen that were 8–9 times that of the R(−) ibuprofen levels at 2 hr plus after oral drug administration compared to the oldest Asian male, which had S(+) levels only 1.5 times greater than R(−) ibuprofen. Male African elephants showed a similar pattern of decreased R(−) concentrations to S(+) concentrations with respect to age, but the difference was less pronounced (1:4 young versus 1:2 old). Serum concentrations measured at 1 hr or more postadministration decreased in a linear fashion when plotted on semilogarithmic graphs (Fig. 1b). These results indicate that R(−) and S(+) ibuprofen were eliminated by first-order kinetics.

Mean and SD pharmacokinetic values are summarized in Table 2. Harmonic mean values were calculated because of the relatively small sample size ($n < 20$). A male Asian elephant was excluded from all statistical calculations because his phar-

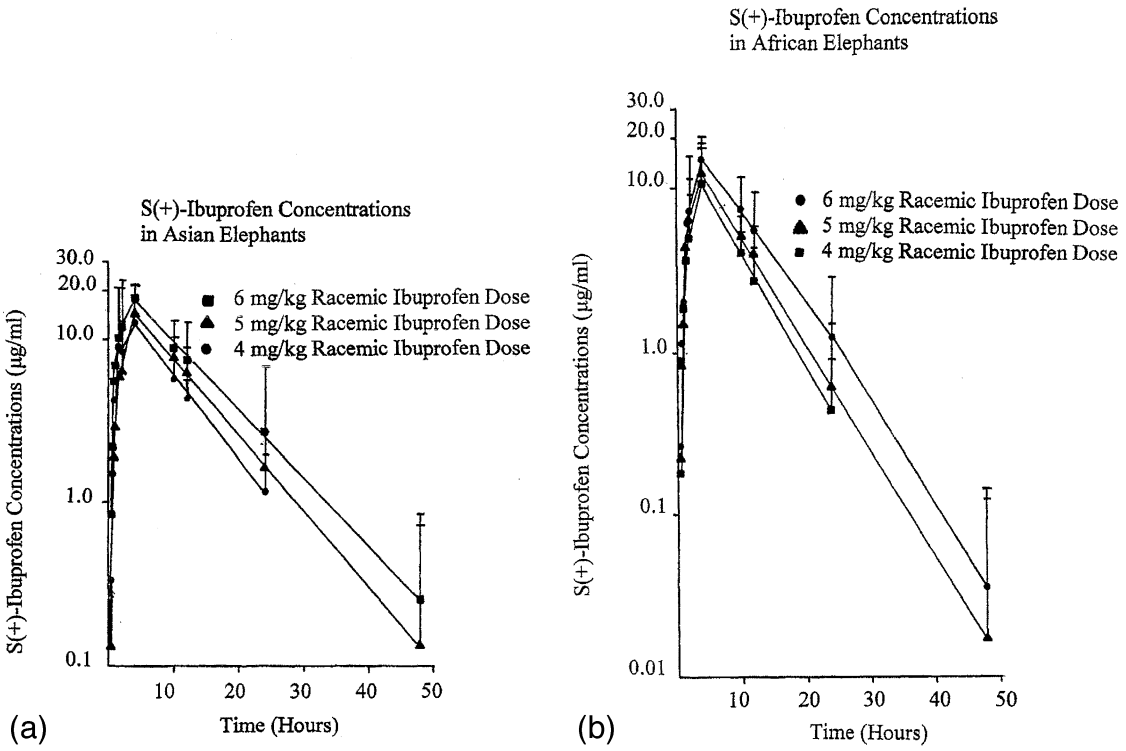

Figure 1. Serum concentrations of R(−) and S(+) ibuprofen enantiomers (mean ± SD) in African and Asian elephants after p.o. administration of 4-, 5-, and 6-mg/kg dosages against time (**a**) and on a semilogarithmic plot to demonstrate elimination by first-order kinetics (**b**).

macokinetic values were outliers most likely associated with advanced age (>50 years old). For example, compared to other Asian elephants in the study, this elephant had a half-life of 53 versus 7.7 hours, a MRT of 79.4 versus 13 hours, and a clearance of 3.2 versus 13 ml/hr/kg for the S(+) enantiomer at the 6-mg/kg oral dose. Racemic ibuprofen was rapidly absorbed. Maximal serum concentrations of ibuprofen were observed in all elephants 4 to 6 hr following p.o. administration, and the T_{max} for both enantiomers of ibuprofen was estimated to be 6.4 ± 3.43 hr for African and 4.7 ± 3.84 hr for Asian elephants for the 6-mg/kg dose. The observed maximal concentration for the 6-mg/kg dosage in African elephants was approximately 5.91 ± 2.17 μg/ml for R(−) and 14.82 ± 9.71 (range: 3.3–24.4) μg/ml for S(+), and was 5.72 ± 1.16 μg/ml for R(−) and 18.32 ± 10.35 (range: 5.1–23.9) μg/ml for S(+) in Asian elephants. The AUC increased in a linear fashion as the dose increased for both enantiomers. The maximal serum concentrations (C_{max}) increased nonlinearly with increasing dosages (4 to 6 mg/kg), but so did the time to reach peak plasma concentrations (T_{max}), which may indicate that the absorption rate was slower at higher doses.

Pharmacokinetic parameter values differed between African and Asian elephants (Table 3). The mean $C_{max,}$ AUC, and $T_{1/2}$ life values for Asian elephants were higher as compared to African elephants, and the mean V_d/F and Cl/F were lower in Asian elephants as compared to African elephants for both S(+) and R(−) ibuprofen enantiomers. The MRT and $T_{1/2}$ for R(−) ibuprofen in Asian and African elephants appeared to be equivalent for all doses given. Serum concentrations of S(+) and R(−) ibuprofen for different dosages visually reflect these general differences between African and Asian elephants (Fig. 2). Multiple dosing trials using racemic ibuprofen 6-mg/kg dosages for Asian and 7-mg/kg dosages for African elephants resulted in similar serum concentrations for both enantiomers of ibuprofen, which were within the therapeutic range (Fig. 3), as well as similar pharmacokinetic parameters (Table 3). The elimination rate constant for 7 mg/kg dosages of ibuprofen in African elephants was 0.259 ± 0.127 hr^{-1} for the R(−) and 0.138 ± 0.097 hr^{-1} for S(+) with a cal-

Table 3. Steady-state chiral pharmacokinetic parameters R(−) and S(+) of ibuprofen following oral multiple dosing to African elephants (7-mg/kg dose every 12 hr) and Asian elephants (6-mg/kg dose every 12 hr).

	African elephants (7 mg/kg)		Asian elephants (6 mg/kg)	
Parameter	R (−)	S(+)	R(−)	S(+)
C_{max} (μg/ml)	6.2 ± 1.27	15.1 ± 2.9	7.0 ± 1.3	17.6 ± 4.0
C_{min} (μg/ml)	4.7 ± 0.60	13.1 ± 1.7	3.5 ± 0.91	9.45 ± 2.64
C_{avg} (μg/ml)	5.17 ± 0.7	13.95 ± 0.9	5.0 ± 1.09	14.1 ± 2.80
T_{max} (hr)	8.80 ± 2.68	10.0 ± 0.45	7.2 ± 4.4	7.0 ± 4.6
V_d (ml/kg)	346.2 ± 112.5	163.1 ± 29.4	299.4 ± 35.2	124.6 ± 14.6
Cl/*F* (ml/hr/kg)	56.4 ± 10.7	20.9 ± 3.9	48.15 ± 10.0	17.8 ± 3.8
Cl/*F* harmonic mean	47.8 ± 19.5	16.9 ± 3.7	46.9 ± 10.0	16.3 ± 3.5
AUC (μg/hr/ml)	62.1 ± 11.7	167.4 ± 30.9	62.4 ± 13.0	168.8 ± 36.1
AUC harmonic mean	64.2 ± 5.37	159.51 ± 31.4	60.3 ± 12.2	173.6 ± 38.6
MRT (hr)	6.14 ± 1.15	7.80 ± 0.63	6.23 ± 0.76	7.00 ± 0.70
K_{el} (hr^{-1})	0.259 ± 0.127	0.138 ± 0.097	0.176 ± 0.058	0.11 ± 0.032
$T_{½}$ (hr)	2.67 ± 2.10	5.0 ± 4.39	3.93 ± 1.47	6.30 ± 1.59

culated half-life of 2.67 ± 2.1 hr for R(−) and 5.0 ± 4.39 hr^{-1} S(+), and for 6-mg/kg dosages in Asian elephants, the K_{el} was 0.176 ± 0.055 hr^{-1} for R(−) and 0.11 ± 0.032 hr^{-1} S(+) with a calculated half-life of 3.93 ± 1.47 hr for R(−) and 6.3 ± 1.59 hr for S(+). The V_d/F for the same respective dosages was estimated to be 346.2 ± 112.2 ml/kg for R(−) and 163.1 ± 29.4 ml/kg for S(+) for African and 299.4 ± 35.2 ml/kg for R(−) and 124.6 ± 14.6 ml/kg for S(+) for Asian elephants.

There appeared to be pharmacokinetic parameter differences between male and female African and Asian elephants (Fig. 4), but they were not statistically significant.

DISCUSSION

The assumed range of therapeutic serum levels of ibuprofen in elephants is based on published ranges for humans. The average dose given to humans is 7 mg/kg, therapeutic serum concentrations range between 15 and 30 mg/L,[26] and the half-life is 2 hr.[1] The S(+) isomer of ibuprofen is reported to be twice as potent as the racemic mixture in its analgesic and anesthetic properties.[2] Although pain studies have not been performed to evaluate the efficacy of ibuprofen or other NSAIDS in elephants, it is assumed that modulation of nociceptive pathways and prostaglandin synthesis are relatively well conserved between placental mammalian species and that ibuprofen's antiinflammatory and analgesic properties are similar in different species. In humans, elimination of total ibuprofen serum concentrations followed nonlinear kinetics, which has been attributed to saturation of plasma protein binding,[11] whereas free drug concentrations followed linear kinetics. In our study, elimination of ibuprofen in elephants followed linear pharmacokinetics. Protein binding of both enantiomers in most species is greater than 99% at therapeutic drug concentrations.[5] This binding is enantioselective and mutually competitive.[8] The R(−) enantiomer binds with a stronger affinity to the same binding sites as the S(+) enantiomer. Because of this enantioselective and competitive protein binding, both R(−) and S(+) enantiomers would have a slight increase in their unbound fraction in the blood, with the S(+) enantiomer having a slightly greater increase in its percentage of unbound drug.[5,8] However, because ibuprofen is so extensively protein bound, this small increase in the free fraction of either enantiomer in the plasma would likely have minimal impact on either enantiomers' volume of distribution (V_d/F). Although there were differences between African and Asian elephants for clearance (Cl/*F*), half-life, and volume of distribution (V_d/f) for all oral doses, they were not statistically significant. The number of animals that would be required to demonstrate statistical differences between the species would range between 19 and 29 for each group, assuming the mean values and variances stayed the same. This study demonstrates that the biological implications of the different trends seen warrant different dosing regimens for the two elephant species. The S(+) enantiomer, with potentially higher levels of unbound drug, might transfer more readily into synovial fluids, where it could elicit greater pharmacological activity. Competitive protein binding between the two enantiomers appears to have little effect on ibuprofen clearance in elephants. Any change in clearance between the two enantiomers when given as a racemic mixture we believe can be attributed to the metabolic or

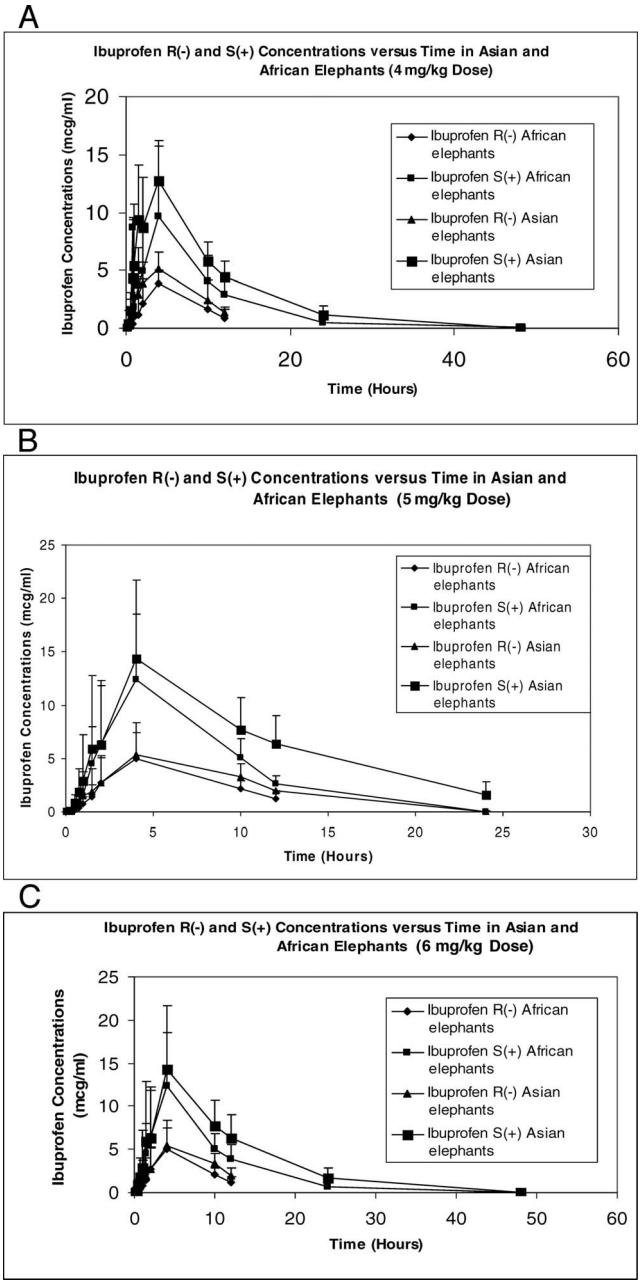

Figure 2. Serum concentrations of R(−) and S(+) ibuprofen after p.o. administration of **A.** 4-, **B.** 5-, and **C.** 6-mg/kg dosages in African compared to Asian elephants against time.

excretion phases (or both) rather than the enantioselective competition of protein binding in systemic circulation.[10]

Using the average weight of elephants in this study and a 70-kg human model given 6 mg/kg p.o. q 6 hr, an allometrically scaled dose of racemic ibuprofen for Asian and African elephants is 2.2 mg/kg given twice daily. This falls within the empirical racemic ibuprofen dosage range of 0.5–4.0 mg/kg s.i.d. (although the dosing frequency is greater), and it is significantly different from the 6 and 7 mg/kg b.i.d. racemic ibuprofen dosage recommendations derived from these research results. Allometrically scaled dosages become less reliable for

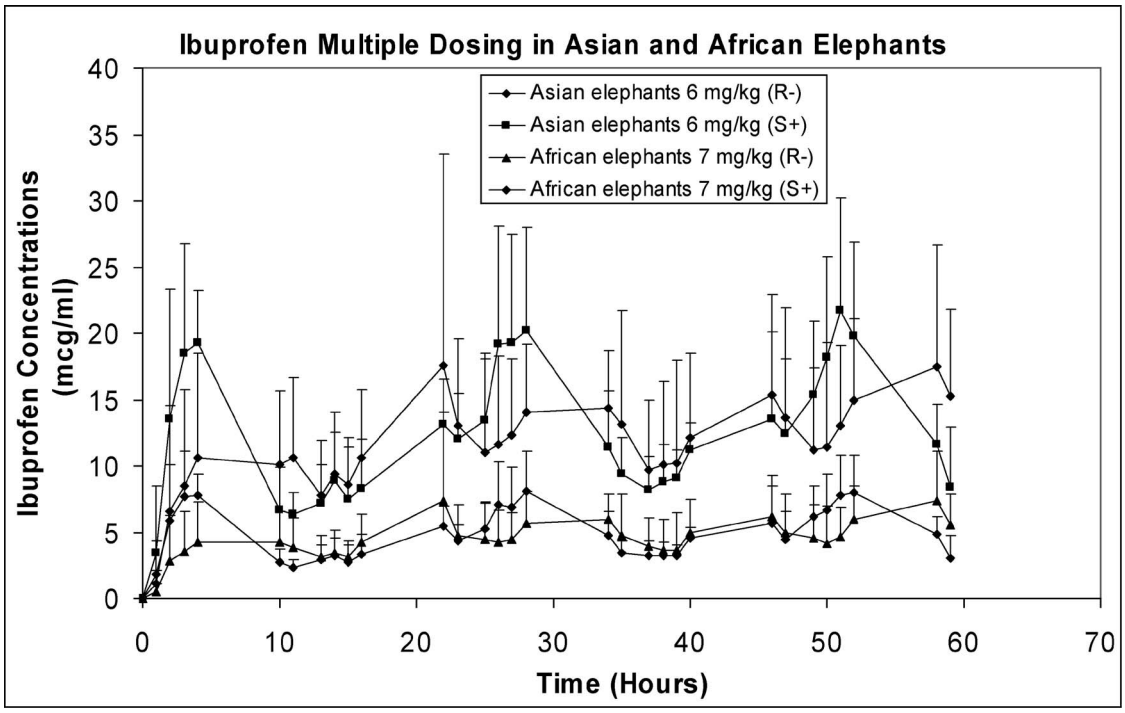

Figure 3. Serum concentrations of R(−) and S(+) ibuprofen after p.o. administration of multiple 6- and 7-mg/kg dosages in Asian and African elephants, respectively, against time.

animals like elephants because the tenets that normally apply no longer hold true (e.g., smaller animals have higher specific basal metabolic rates based on more rapid mean circulation times, higher densities of capillaries per unit of tissue, greater respiratory gas exchange surface areas, higher glomerular filtration rates, and greater surface areas per unit of body size).[6,25]

A significant difference was seen in the dosage of racemic ibuprofen required to achieve and maintain therapeutic serum concentrations between African and Asian elephants. African and Asian elephant species differ in a number of ways morphologically, including height, weight, ear size, distal trunk processes, number of digits per foot, number of ribs, and number of caudal vertebrae. Differences in pharmacokinetic parameters for various drugs between these two species have been demonstrated for both antibiotic (e.g., trimethoprim–sulfamethoxazole)[18] and anesthetic agents (over three times the dose of ketamine is required for immobilization of African versus Asian elephants).[14] The disparity in the dosages between African and Asian elephants determined in this study cannot be predicted by allometrically scaling the pharmacokinetically derived dose of one species to another. In fact, the difference in the reported dosage requirement is more likely indicative of an underlying metabolic difference in the uptake, distribution and/or elimination of ibuprofen between the two species, because the average body weight for the Asian elephant group was greater (3961 ± 677 kg) than the African elephant group (3573 ± 1025 kg). Indeed, the AUC, $T_{1/2}$ life, mean Cl, and K_{el} values reflect a greater distribution and slower elimination of ibuprofen in Asian as compared to African elephants. In humans, ibuprofen undergoes significant chiral conversion of R(−) enantiomer to the S(+) enantiomer, which is eliminated following biotransformation to glucuronide conjugate metabolites that are excreted in urine, with little of the drug being eliminated unchanged, and this is most likely the case for elephants as well.[9] Twenty-four hours after the racemic ibuprofen mixture was given orally to both Asian and African elephants, no R(−) ibuprofen was detectable in serum samples. The R(−) isomer had a significantly lower $T_{1/2}$ and a faster rate of elimination, suggesting that chiral conversion of ibuprofen from the R(−) to S(+) enantiomer occurs in both Asian and African elephants. However, it could also be that the R(−) enantiomer is simply eliminated more rapidly.

Additional factors potentially affecting dosage requirements in these elephants include general

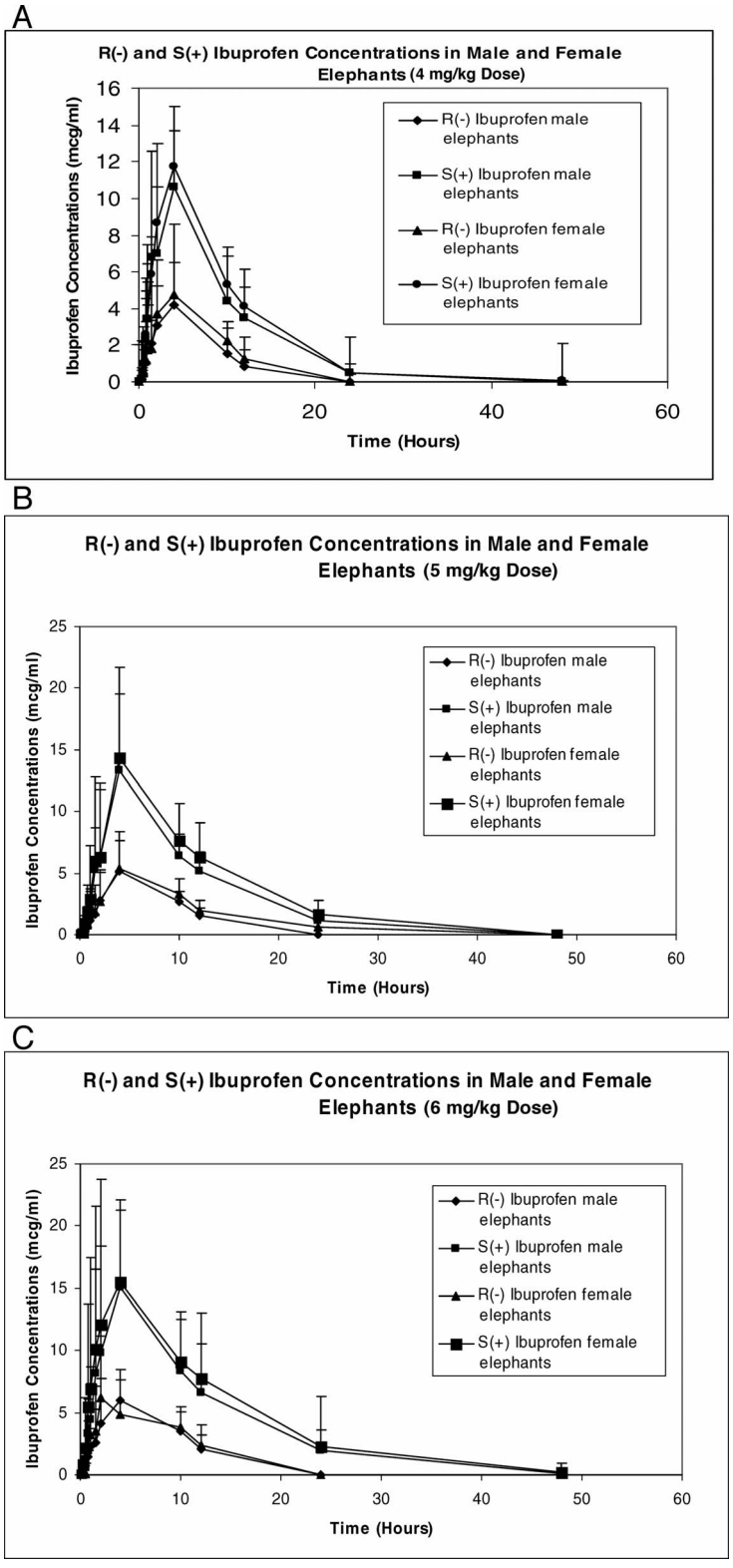

Figure 4. Serum concentrations of R(−) and S(+) ibuprofen (mean ± SD) in male versus female African and Asian elephants after p.o. administration of **A.** 4-, **B.** 5-, and **C.** 6-mg/kg dosages against time.

health, age, and gender. All of the animals included in this study were in good general health, and males were not included during periods of musth. The African elephants were comparatively young, ranging in age from 18 to 25 years (mean 21.4) versus the Asian elephants, which were between 13 and 43 years old (mean 31.4), and this difference was statistically significant ($p < 0.008$). Younger animals typically function at a higher metabolic rate,[22] so we cannot preclude the potential effect that age had on metabolic rate and subsequent pharmacokinetic parameter calculations in this study. However, even after accounting for the difference in age between African and Asian elephants, pharmacokinetic parameters were still different between these two groups. For example, Cl/*F* values for 6-mg/kg dosages for African elephants were 66.4 ml/hr/kg R(−) and 20.1 ml/hr/kg S(+), compared to Asian elephants, with 62.5 ml/hr/kg R(−) and 13.8 ml/hr/kg S(+). Asian elephants of similar age to African elephants in the study had clearance values of 66.5 R(−) and 18.5 S(+) ml/hr/kg for 6-mg/kg dosages. Therefore, age appears to have an effect on clearance independent of species differences, as determined by regression analysis; however, age did not fully account for differences seen in the pharmacokinetic parameters between Asian and African elephants. Similar findings were observed for every pharmacokinetic parameter calculated for similarly aged Asian and African elephants.

There appeared to be an overall trend indicating that Cl/*F* decreases with age, so it might be prudent to lower dosages or decrease dosing frequency in older elephants. Elephants in the advanced elderly stage of life should be given no more than 2 mg/kg once daily of ibuprofen (based on observations of serum concentrations from two elephants only).

Differences in drug metabolism may also be based on gender, with males typically requiring higher dosages than females.[24] Although nothing has yet been published about elephants in this regard; there is no reason to believe that they differ in this regard from other mammalian species. In this study, female African and Asian elephants generally appeared to have lower dosage requirements as compared to males of the same species (Fig. 4). For example, for 6-mg/kg dosages, the AUC for female African elephants was 49.0 ± 21.0 μg/hr/ml R(−) and 161.4 ± 71.0 μg/hr/ml S(+) and for males it was 48.6 ± 37.7 R(−) and 156.4 ± 147.1 μg/hr/ml, and the $T_{1/2}$ for females was 2.49 ± 0.8 hr R(−) and 5.70 ± 1.16 hr S(+), and for males it was 2.3 ± 0.38 hr R(−) and 5.9 ± 3.66 hr S(+). This comparison exemplifies a potential metabolic difference based on gender in the uptake, distribution, and/or elimination of ibuprofen. Gender differences among elephants appear to be biologically important, although a larger sample size will be required to demonstrate statistical significance. Also, this difference in gender may be more important in young animals, as the elimination of the R(−) enantiomer or conversion of R(−) to S(+) ibuprofen is greater in younger males and less in older males compared to similarly aged females. Again, slightly lower dosages for female elephants might provide therapeutic serum concentrations of ibuprofen.

Diurnal variations in drug metabolism have been documented for antiinflammatory agents.[26] For example, peak serum concentrations of naproxen in humans were delayed when the same dose was administered orally in the morning versus evening hours.[19] This is likely associated with diurnal variations in serum concentrations of cortisol, which are typically higher in the morning in elephants as well (Bechert, unpubl. data). The logistic challenge of coordinating sample collections among a variety of zoos and private facilities precluded an investigation into diurnal variations of drug metabolism for our study. However, veterinarians are encouraged to consider potential variations in drug metabolism, and therefore efficacy, with respect to time and method of administration.

In conclusion, the therapeutic dose of racemic ibuprofen for African elephants appears to be 7 mg/kg given every 12 hr and 6 mg/kg for Asian elephants, also given every 12 hr. Larger sample sizes are required to confirm the differences observed relative to gender and advanced age. Although no side effects were seen during this study, long-term administration of ibuprofen was not conducted, so confirmation of the safety of ibuprofen 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 the possible effects of advanced age on the metabolism of ibuprofen, and to monitor hepatic and renal function during the course of long-term ibuprofen administration.

Acknowledgments: The authors would like to thank the elephant keeper staff at the Kansas City Zoo, Riddle's Elephant Sanctuary, the Bowmanville Zoo, Pittsburgh Zoo, Have Trunk Will Travel, and Oregon Zoo for their assistance in collecting blood samples for this study. Funding for this research was provided by The Morris Animal Foundation.

LITERATURE CITED

1. Bapuji, A. T., D. Rambhau, P. Srinivasu, B. R. Rao, and S. S. Apte. 1999. Time-dependent influence of diaz-

epam on the pharmacokinetics of ibuprofen in man. Drug Metab. Drug Interact. 15: 71–81.

2. Bonabello, A., M. R. Galmozzi, R. Canapara, G. C. Isaia, L. Serpe, E. Muntoni, and G. P. Zara. 2003. Dexibuprofen S(+) isomer of ibuprofen reduces gastric damage and improves analgesic and anti-inflammatory effects in rodents. Anesth. Analg. 97: 402–408.

3. Garcia, R. 1998. Variability in risk of gastrointestinal complications with different nonsteroidal anti-inflammatory drugs. Am. J. Med. 104: 30–34.

4. Gulland, F. M. D., and P. C. Carwardine. 1987. Plasma metronidazole levels in an Indian elephant (*Elephas maximus*) after rectal administration. Vet. Rec. 120: 440.

5. Hao, H., G. Wang, and J. Sun. 2005. Enantiomer-selective pharmacokinetics of ibuprofen and involved mechanisms. Drug Metab. Rev. 37: 215–234.

6. Hayssen, V., and R. C. Lacy. 1985. Basal metabolic rates in mammals: taxonomic differences in the allometry of BMR and body mass. Comp. Biochem. Physiol. 81A: 741–754.

7. Hunter, R. P., R. Isaza, and D. E. Koch. 2003. Oral bioavailability and pharmacokinetic characteristics of ketoprofen enantiomers after oral and intravenous administration in Asian elephants (*Elephas maximus*). Am. J. Vet. Res. 64(1): 109–114.

8. Itoh, T., J. Maruyama, Y. Tsuda, and H. Yamada. 1997. Stereoselective pharmacokinetics of ibuprofen in rats: effect of enantiomer–enantiomer interaction in plasma protein binding. Chirality 9: 354–361.

9. Jamali, F., N. N. Singh, F. M. Pasutto, A. S. Russell, and R. T. Coutts. 1988. Pharmacokinetics of ibuprofen enantiomers in humans following oral administration of tablets with different absorption rates. Pharm. Res. 5: 40–43.

10. Lin, W., T. Hayakawa, H. Yanaguimoto, M. Kuzuba, T. Obara, G. Ding, F. Cui, and N. Inotsume. 2004. Pharmacokinetic interaction of ibuprofen enantiomers in rabbits. J. Pharm. Pharmacol. 56: 317–321.

11. Lockwood, G. F., K. S. Albert, and W. R. Gillespie. 1983. Pharmacokinetics of ibuprofen in man: free and total area/dose relationships. Clin. Pharmacol. Ther. 34: 97–103.

12. Lockwood, G. F., and J. G. Wagner. 1982. High-performance liquid chromatographic determination of ibuprofen and its major metabolites in biological fluids. J. Chromatogr. 232: 335–343.

13. Lodwick, L. J., J. M. Dubach, L. G. Phillips, C. S. Brown, and M. A. Jandreski. 1994. Pharmacokinetics of amikacin in African elephants (*Loxodonta africana*). J. Zoo Wildl. Med. 25: 367–375.

14. Marx, K. L., and M. A. Roston. 1996. The Exotic Animal Drug Compendium. Veterinary Learning Systems, Trenton, New Jersey. P. 62.

15. Mikota, S. K., E. L. Sargent, and G. S. Ranglack. 1994. Medical Management of the Elephant. Indira Publishing House, West Bloomfield, Michigan. Pp. 137–150.

16. Mortenson, J., and S. Sierra. 1998. Determining dosages for anti-inflammatory agents in elephants. Proc. Am. Assoc. Zoo Vet. Pp. 477–479.

17. Niesen-Kudsk, F. 1980. HPLC determination of some anti-inflammatory, weak analgesic and uricosuric drugs in human blood plasma and its applications to pharmacokinetics. Acta Pharmacol. Toxicol. 47: 267–273.

18. Page, C. D., M. Mautino, H. D. Derendorf, and J. P. Anhalt. 1991. Comparative pharmacokinetics of trimethoprim–sulfamethoxazole administered intravenously and orally to captive elephants. J. Zoo Wildl. Med. 22: 409–416.

19. Rao, B. R., D. Rambhau, and W. Roa. 1993. Pharmacokinetics of single-dose administration of naproxen at 10:00 and 22:00 h. Chronobiol. Int. 10: 137–142.

20. Rosin, E., N. Schultz-Darken, B. Perry, and J. A. Teare. 1993. Pharmacokinetics of ampicillin administered orally in Asian elephants (*Elephas maximus*). J. Zoo Wildl. Med. 24: 515–518.

21. Schmidt, M. J. 1978. Penicillin G and amoxicillin in elephants: a study comparing dose regimens administered with serum levels achieved in healthy elephants. J. Zoo Anim. Med. 9: 127–136.

22. Sedgewick, C. J. 1993. Allometric scaling and emergency care: the importance of body size. *In:* Fowler, M. E. (ed.), Zoo and Wild Animal Medicine, 3rd ed. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 34–37.

23. Siedeman, P., F. Lohrer, G. Graham, M. W. Duncan, K. M. Williams, and R. O. Day. 1994. The stereoselective disposition of the enantiomers of ibuprofen in blood, blister and synovial fluid. Br. J. Clin. Pharmacol. 38: 221–227.

24. Walker, J. S., and J. J. Carmody. 1998. Experimental pain in healthy human subjects: gender differences in nociception and in response to ibuprofen. Anesth. Analg. 86: 1257.

25. West, G. B., J. H. Brown, and B. J. Enquist. 1997. A general model for the origin of allometric scaling laws in biology. Science 276: 122–126.

26. White, S., and S. H. Y. Wong. 1998. Standards of laboratory practice: analgesic drug monitoring. Clin. Chem. 44: 1110–1123.

Received for publication 18 September 2006