SERUM OXYTETRACYCLINE CONCENTRATIONS IN AFRICAN ELEPHANT (*LOXODONTA AFRICANA*) CALVES AFTER LONG-ACTING FORMULATION INJECTION

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Abstract: Serum oxytetracycline pharmacokinetics were studied in 18 African elephant (*Loxodonta africana*) calves. Each elephant received separate injections of oxytetracycline at approximately 18 mg/kg i.m. and 8 mg/kg i.v. in a cross-over study. Blood samples were drawn at 0, 24, 48, 72, and 96 hr postinjection. An additional sample was drawn 110 hr before the animals were reinjected in the cross-over study and a final blood sample was drawn 48 hr after the second dose. No lameness or stiffness was observed following i.m. injections. Serum oxytetracycline concentrations >0.5 μg/ml were present 48 hr after initial dosing for all elephants (i.m., i.v., high or low dosage). Only elephants given the high i.m. dosage (18 mg/kg) maintained levels >0.5 μg/ml 72 hr postinjection. No significant difference in serum oxytetracycline concentration with time was observed between the groups given different i.v. dosages. These studies demonstrated that quantifiable serum oxytetracycline concentrations can be maintained in young African elephants with a low-dosage multidose i.m. regimen.

Key words: African elephant, *Loxodonta africana*, oxytetracycline, serum drug concentrations, pharmacokinetics.

INTRODUCTION

Tetracyclines are bacteriostatic, with bactericidal effects at high concentration. They have broad activity against gram-negative and gram-positive bacteria, mycoplasma, chlamydia, borrelia, many rickettsia, and ameba.[5,9,11] Tetracyclines have large volumes of distribution and a relatively long half-life of elimination, and they diffuse into most body fluids and tissues.[9] These characteristics, combined with relatively low cost, have made them highly attractive for therapeutic use in wildlife. Proper dosing is important, however, because of the potential for development of microbial resistance.[5,10,14,21]

Dosages of oxytetracycline (OTC) >60 mg/cm of combined length and girth measurement, delivered i.m. or i.v., provide therapeutic serum concentrations for at least 48 hr in adult male African elephants (*Loxodonta africana*),[4] and the drug is therefore potentially useful in that species. Dosages comparable to those established for cattle and horses have been recommended for young elephants, but 25–50% lower dosages have been recommended for adult elephants.[20] The issue of appropriate scaling of drug dosages for elephants remains controversial, particularly for young animals.[15,16,18] The use of routine approaches to scaling by weight or

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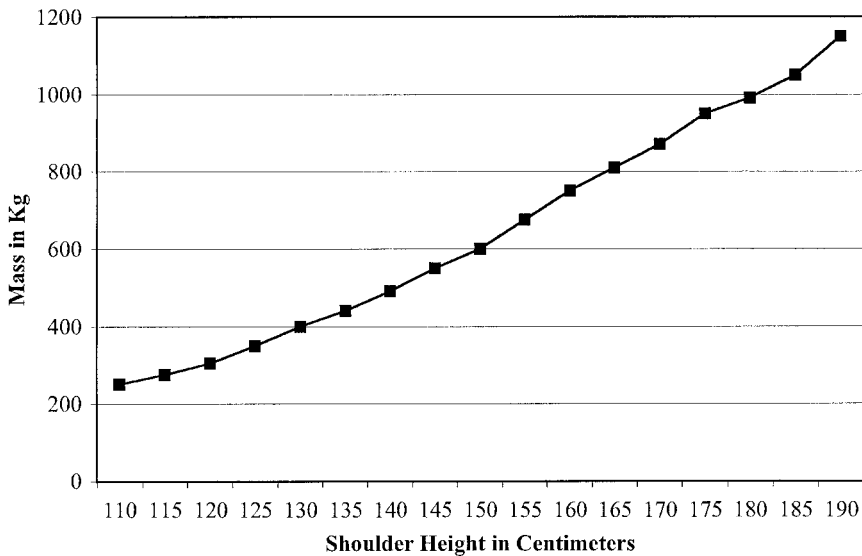
metabolism is further complicated because although the main route of excretion of oxytetracycline in mammals is through renal glomerular filtration, the drug is also excreted through bile.

In this study, we examined the pharmacokinetics of long-acting OTC formulations in African elephant calves following single injections.

MATERIALS AND METHODS

Serum OTC pharmacokinetics were studied in 18 African elephant calves held for a tuberculosis survey and encephalomyocarditis vaccine trial in Kruger National Park, South Africa.[8] The estimated weights of the elephants were 600–980 kg, based on shoulder height data collected for several years in Kruger National Park from elephant calves that were subsequently weighed (Fig. 1, Raath, unpub. data). The animals were housed in groups of three in bomas constructed of steel cables and were fed leucerne hay and fresh local browse cut daily and fed ad lib.

Each elephant was assigned to a group in no particular order and received long-acting OTC injections (200 mg/ml, Tetravet 20% LA, Kruger-Med Pharmaceutical Ltd., Park Central 2001, South Africa) at either 18 mg/kg i.m. or 8 mg/kg i.v. The i.v. doses were administered via 16-ga catheters (Intracath, Deseret Co., Sandy, Utah 84070, USA) in an ear vein. The i.m. injections were made mainly in the triceps and gluteal muscles using a 7.6-cm 18-ga needle. No more than 15 ml were injected in a single site.

Blood samples were drawn from the ear vein prior to drug administration and at 24-hr intervals over 96 hr. At 96 hr, all serum OTC levels were expected

Figure 1. Semilog plot of weight (kg) measured for young African elephants (*Loxodonta africana*) from Kruger National Park versus their shoulder height measured in centimeters. Each point represents a single measurement.

to be below levels of possible quantitation. A crossover study design was employed. An additional sample was drawn at 110 hr as a baseline, and the animals were reassigned to groups in no particular order and reinjected with OTC, again either 18 mg/kg i.m. or 8 mg/kg i.v. A final blood sample was drawn for analysis 48 hr after the second antibiotic injection.

The elephant calves were anesthetized for the antibiotic injections and for the serial blood collections. Anesthesia was induced using etorphine HCl (M99, 9.8 mg/ml, C-Vet, Minster House, Bury St. Edmunds, Suffolk, UK; 1.5 mg i.m.) combined with azaperone (Stresnil, Wildlife Pharmaceuticals, Fort Collins, Colorado 80524, USA; 20 mg i.m.) in a 3-ml plastic dart with a 2-mm-diameter, 60-mm-long noncollared needle delivered by compressed CO_2 rifle (Dan-inject, Wildlife Pharmaceuticals). The three calves in a boma were anesthetized at the same time, and usually showed signs of anesthetic effects within 10 min of injection and lateral recumbency within 15 min. The antibiotic injections were given with the calves in lateral recumbency, but most blood samples were obtained from standing narcotized calves, prior to recumbency. After all three calves in a boma were treated or sampled, each was given naltrexone HCl (Trexonil, Wildlife Pharmaceuticals, 60 mg i.v.) to reverse the narcotic effects, and each returned to normal activity within 5–10 min. No mortality or morbidity was associated with the seven anesthetic procedures performed on each calf.

Blood samples were cooled on ice (4°C) and centrifuged within 4 hr of collection, and the serum was separated and stored at −70°C until assayed. Serum concentrations of OTC were determined by high-performance liquid chromatography using a method developed for tetracyclines and modified for analysis of elephant serum.[13] Calibration curves were prepared daily and were linear with an r^2 value of at least 0.99, with back calculation of calibration standards within 15% of true value. Ten different concentrations (0.25–100 µg/ml) of OTC in serum from untreated elephants, prepared using a stock solution of 1 mg/ml OTC in distilled water, were used to establish the calibration curve. Untreated elephant serum served as the blank. Injection volume was 50 µl. The limit of detection was 0.125 µg/ml, and the limit of quantitation (LOQ) was 0.25 µg/ml.

Pharmacokinetic parameters were calculated using the following formulas:[1,19] $t_{1/2} = 0.693/\beta$, where $t_{1/2}$ is the disposition half-life and β is the slope of the disposition curve; dose = clearance × AUC, where AUC is the area under the serum concentration curve; $Vd_{area} = \text{dose}/\text{AUC} \times \beta$, where Vd_{area} is the volume of distribution calculated by the area method and β is the slope of the elimination phase of the disposition curve; and $F = \text{AUC}_{i.m.}/\text{AUC}_{i.v.} \times \text{dose}_{i.v.}/\text{dose}_{i.m.}$, where F is the availability. Data from both i.m. and i.v. administrations were fitted using a one-compartment model characterized by the exponential equation $C = C_i e^{-\beta t}$, where C is the concentration, C_i is the extrapolated zero-time se-
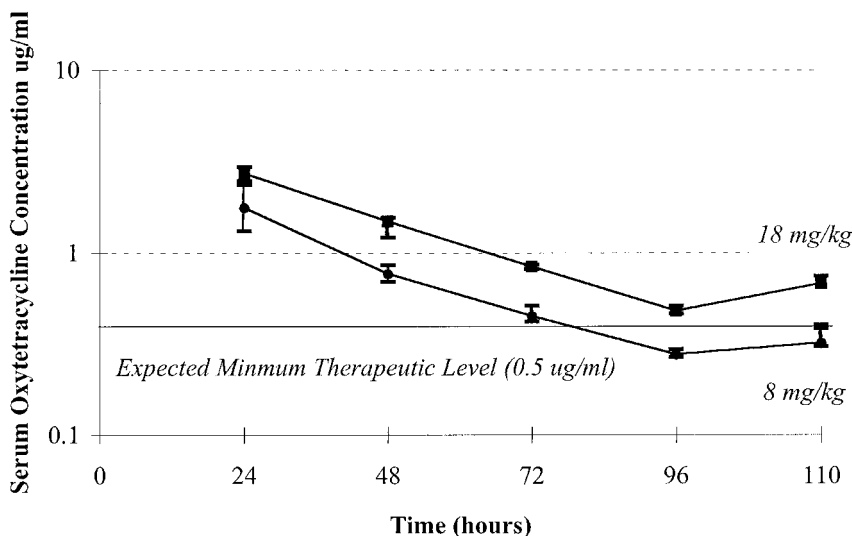
Figure 2. Semilog plot of serum concentration of oxytetracycline in African elephant calves (*Loxodonta africana*) administered approximately 8 mg/kg (*n* = 4) or 18 mg/kg (*n* = 5) oxytetracycline i.m. versus time (hr). Minimum concentration expected to have therapeutic potential is 0.5 μg/ml.

rum drug concentration intercept, e is the natural log, β is the slope of the disposition curve calculated using the serum OTC concentrations obtained from the 24-, 48-, 72-, 96-, and 110-hr samples.

RESULTS AND DISCUSSION

All of the elephants used in this trial were well within the appropriate size range for young calves. Calculations of the animals' weights based on a semilog plot of shoulder height to weighted mass of other animals (Fig. 1) showed that the calves placed in the high-dosage i.m. trials were significantly larger than those in the other treatment groups. This difference in weight had no obvious impact on the results of the study because each animal was dosed based on individually estimated weight, and pharmacokinetic calculations were performed for each elephant, reducing the impact of actual dose variability.

Some error between actual and estimated dose based on weight is expected when morphometric estimators are used. Although the actual delivered dosage in high-dosage trials was remarkably the same for both the i.m. and the i.v. groups, the actual dosages used in the low-dosage trials were not equal. A higher i.v. dosage was administered. This difference was also taken into account by calculating the pharmacokinetic parameters of each individual animal based on actual dose administered and then evaluating the means of those values to assess the treatment of the group.

No lameness or stiffness was observed in ani-

mals administered i.m. injections nor was there any evidence of muscle swelling or local heat at the i.m. injection sites when examined at 24-hr intervals through 96 hr postinjection. The detected serum concentrations of OTC, plotted against time for i.v. and i.m. routes, are shown in Figures 2 and 3, respectively. Pharmacokinetic parameters are reported in Table 1.

The minimum inhibitory concentration (MIC) of OTC varies for different target organisms and can change over time because of local antimicrobial drug utilization,[12,22] but studies in Nigeria and Zimbabwe suggest that strains of common pathogenic bacteria isolated from African animals are more sensitive to OTC, having lower MICs than found for the same bacteria species in more developed countries such as the United States or Australia.[3,6] Serum OTC concentrations > 0.5 g/ml are routinely considered therapeutic for a range of bacteria, including *E. coli, Bacillus* spp., and *Acintobacter* spp.; however, higher MICs are routinely found even in Africa for *Pasteurella hemolytica* and *Staphylococcus* spp.[6,7] In our study, serum concentrations >0.5 μg/ml were present 48 hr after initial dosing for all elephants (i.m., i.v., high or low dosage). Only elephants given the high i.m. dosage maintained levels >0.5 μg/ml at 72 hr postinjection. No difference in serum concentration with time was observed between low and high i.v. dosages.

The LOQ in elephant serum in this study was 0.25 μg/ml. Serum concentrations in all elephants
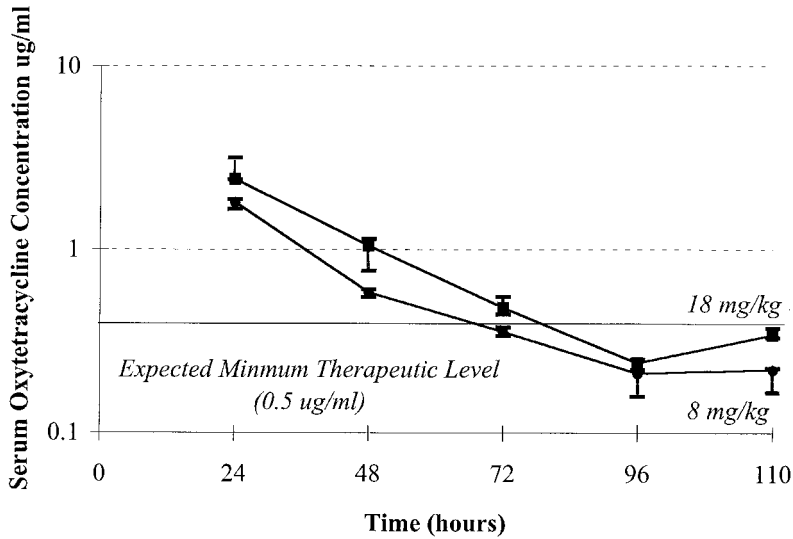
Figure 3. Semilog plot of serum concentration of oxytetracycline in African elephant calves (*Loxodonta africana*) administered approximately 8 mg/kg (*n* = 4) or 18 mg/kg (*n* = 5) oxytetracycline i.v. versus time (hr). Minimum concentration expected to have therapeutic potential is 0.5 µg/ml.

in the i.m. trials and those in the high-dosage i.v. trials remained above the LOQ for 110 hr. This would have been expected to contribute to elevated levels in the second injection trial; however, mean serum concentrations 48 hr after the second injection for each dosage and administration route never exceeded those detected 48 hr after the first injection. Mean serum concentrations 48 hr after second OTC injection for 8 mg/kg i.m., 18 mg/kg i.m., 8 mg/kg i.v., and 18 mg/kg i.v. were 87%, 82%, 84%, and 60.5%, respectively, of mean serum concentrations 48 hr after the first injection.

A slight upturn in the serum OTC concentration curves at the 110-hr time point was consistent among all four groups of elephants. In the case of the i.v. high-dosage group, this increase brought the values above the LOQ, suggesting that the effect may not be simply a coincidental error caused by random noise in the assay of very low serum concentrations of drug. This slight upturn may be due to late release of drug from a minor depot site. Alternatively, it may reflect enterohepatic recirculation from the fraction of drug being excreted in the bile into the gastrointestinal system.

The differences observed in clearance between treatment groups were not statistically significant,

Table 1. Pharmacokinetic parameters ($\bar{x} \pm$ SD) of oxytetracycline administration in African elephant (*Loxodonta africana*) calves.

| | Dose (mg/kg) | | | |
| | Intramuscular | | Intravenous | |
Parameter	8.6	18.2	9.2	18.2
n	4	5	4	4
Shoulder height (cm)	158.5 ± 5.6	171.2 ± 5.6	165.0 ± 3.7	161.5 ± 5.9
Estimated weight (kg)	718 ± 76	904 ± 56	819 ± 55	756 ± 78
β (hr^{-1})	0.028 ± 0.013	0.023 ± 0.002	0.036 ± 0.001	0.039 ± 0.003
$t_{1/2}$ (hr)	28.8 ± 9.9	30.9 ± 2.7	19.2 ± 0.3	18.1 ± 1.3
AUC$_{0 \to \infty}$ (µg·hr/ml)	135 ± 57	181 ± 21	106 ± 18	183 ± 33
Clearance (ml/min/kg)	75.8 ± 31.4[a]	101.8 ± 12.3[a]	89.2 ± 18	102.8 ± 16.8
Vd (ml/kg)	3,556 ± 2,218[a]	4,566 ± 879[a]	2,481 ± 538	2,686 ± 475
F (%)	136.2	98.9		

[a] Values are for comparison purposes only. Because absorption after i.m. administration cannot be considered complete these values cannot be accurately calculated.

although there was more variability in the group receiving the i.m. low dosage. These values were calculated for comparison only, because absorption after i.m. administration cannot be considered complete and the site of injection can dramatically affect data. The calculated volume of distribution was most variable in the i.m. low-dosage group and generally more variable in the i.m. trials, whereas actual clearance values were significantly lower in the i.v. trials. This result was expected, considering the potential effect of administration of the drug into more or less vascular sites and an expected delay in transition from deep muscle tissue to the vascular compartment. However, bioavailability from i.m. injection appeared to be essentially complete.

Drug scaling between calves and adult elephants is an important issue. Unfortunately, the only similar work in adult African elephants reported OTC doses in relationship to length plus girth measurements in centimeters, with no relationship to mass or body weight determined.[4] In this study, shoulder height was used as a mass estimator with the correlation to body mass provided in Figure 1. Therefore, careful assessment of scaling issues between calves and adults is not possible. However, serum OTC values 48 hr postinjection did not vary with dose in either this study or the earlier study with adult elephants. The 48-hr serum values from calves receiving OTC i.v. in this study were approximately double the values seen in adult elephants administered an unknown i.v. dose. A trend toward differences in serum OTC values related to dose may be present in i.m. trials in adult elephants,[4] but the range is narrow and the small number of elephants involved precludes conclusive interpretation. In this study a dose-dependent difference in serum OTC values was apparent in calves administered the drug i.m., and those values ranged around the values reported in the previous work with adult elephants.

These studies demonstrated that serum OTC concentrations can be maintained at quantifiable levels in young African elephants with a multidose low-dosage i.m. regimen. At no time in this study, however, was a serum concentration >4 μg/ml, as recommended by the National Committee for Clinical Laboratory Standards, detected. It will be extremely important to examine the efficacy of OTC against specific pathogens isolated from elephants, because the range of susceptibility to tetracycline varies greatly among genera, species, and strains of bacteria.[3,6,12,22] Increasing the i.v. dosage more than two-fold in these studies did not increase serum concentrations of the drug. The elimination half-lives determined for the two different i.m. dosage trials were statistically indistinguishable. These values were nearly double the half-lives calculated for the i.v. trials, which were also apparently independent of dosage in the range examined in this study.

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