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Author(s): Aondover F. Gberindyer, DVM, MSc, Ene R. Okpeh, DVM, and Asaaga A. Semaka, DVM, MVSc

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Pharmacokinetics of Short- and Long-acting Formulations of Oxytetracycline After Intramuscular Administration in Chickens

Aondover F. Gberindyer, DVM, MSc, Ene R. Okpeh, DVM, and Asaaga A. Semaka, DVM, MVSc

Abstract: Both short- and long-acting formulations of oxytetracycline are commonly used in veterinary medicine to treat animals infected with gram-negative and gram-positive bacteria, rickettsiae, mycoplasma, and chlamydiae. To compare pharmacokinetics of short- and long-acting oxytetracycline in chickens, injectable formulations from the same pharmaceutical company were administered to healthy 6-week-old broiler chickens in accordance to the labeled instructions. Fourteen chickens were separated into 2 groups: chickens in group A (n = 7) were administered the short-acting formulation (10 mg/kg IM q24h) for 4 consecutive days, whereas those in group B (n = 7) were treated with a single dose (20 mg/kg IM) of the long-acting formulation. Blood samples were collected into heparinized tubes before and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, and 24 hours after initial treatment. Thereafter, blood samples were taken every 24 hours up to 120 hours. Plasma concentrations of oxytetracycline were determined by competitive enzyme-linked immunoabsorbent assay, and pharmacokinetic parameters were obtained. Both formulations delivered therapeutic plasma concentrations of oxytetracycline for approximately 100% of their respective dosing intervals as recommended. However, considering the additional labor, patient stress, and mortalities associated with handling, in addition to rejection of the carcass due to tissue necrosis resulting from multiple injections, we recommend use of the long-acting instead of the short-acting injectable formulation in broiler chickens.

Key words: oxytetracycline, long-acting oxytetracycline, short-acting oxytetracycline, pharmacokinetics, avian, chickens

Introduction

Oxytetracycline, a tetracycline isolated from *Streptomyces rimosus*, is widely used in veterinary medicine against gram-positive and gram-negative bacterial organisms, rickettsiae, mycoplasma, and chlamydiae.[1] Short- and long-acting, injectable formulations are commonly used at the dose rate of 10 mg/kg and 20 mg/kg, respectively. The long-acting formulation is usually administered once and is expected to maintain a therapeutic plasma concentration for 3 to 4 days, whereas the short-acting formulation is recommended for daily use for 3 to 4 days to maintain a therapeutic plasma concentration.[2] Results of a previous study[3] indicated that the long-acting formulation gives lower peak but longer periods of therapeutic plasma concentration when compared with the short-acting formulation administered once.

Oxytetracycline administration via intramuscular (IM) or subcutaneous (SC) injection circumvents the shortcoming of unreliable intake in poultry when administered via drinking water. Intramuscular administration of drugs, however, often causes discomfort to the animal and requires additional labor from the veterinarian in animal handling and the number of visits to the farm, resulting in an additional financial cost to the client. Therefore, the design of a long-acting formulation of oxytetracycline has circumvented these shortcomings. However, frequent handling and visits to the farm also give the veterinarian a

From the Departments of Veterinary Physiology, Pharmacology and Biochemistry (Gberindyer, Okpeh), and Veterinary Medicine, Avian Unit (Semaka), University of Agriculture, PMB 2373, Makurdi, Benue State 970001, Nigeria.

better opportunity to assess the effect of the therapeutic intervention. There is a paucity of information on the plasma concentration profiles of oxytetracycline in chickens following IM treatment with a therapeutic dose of short-acting oxytetracycline compared with the long-acting form.

Consequently, this study was designed to compare the pharmacokinetics of short- and long-acting formulations of oxytetracycline from the same manufacturer in chickens treated in accordance to the manufacturer's recommendations.

Materials and Methods

Drugs

Short- and long-acting oxytetracycline injectable formulations (Divine Laboratory Services, Gujarat, India) with label strength of 50 mg/mL and 200 mg/mL, respectively, were used.

Animals

Six-week-old, clinically healthy, mixed sex broiler chickens, weighing between 0.8 and 1.1 kg and from the same hatchery, were used in this study. During the 1-week period of acclimatization, birds were given access to nonmedicated water ad libitum and fed a drug-free commercial growers' marsh. Experimental animals were handled in the course of study in accordance with animal care and welfare committee regulations of the College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria.

Study design

A parallel design was adopted in which animals were randomly assigned to groups A and B ($n = 7$ each group). Chickens in group A were treated daily for 4 days with short-acting oxytetracycline at a dose rate of 10 mg/kg IM (pectoral muscle). Whereas those in group B were given long-acting oxytetracycline at a single dose of 20 mg/kg IM. These doses were based on the manufacturer's recommendation and the common practice in Nigeria. Before and after the experiment, blood samples (1 mL) were obtained from the experimental chickens for individual hematologic profiles as required by the college research ethical committee. On day 1 before treatment and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, and 24 hours after approximately 0.5 mL of blood was obtained via either the jugular or brachial veins from each animal and placed into lithium heparinized tubes. Thereafter, blood samples were similarly obtained from all chickens every 24 hours up to 120 hours after the initial treatment. For chickens in group A, the 24-hour interval sampling was done 5 minutes before each daily treatment. The interval between assigned sampling point and the actual time of sampling was not more than 3 minutes for all chickens.

Blood samples were subsequently centrifuged at 2800*g* for 5 minutes and the supernatant (plasma) was obtained with a micropipette. The plasma was preserved at –20°C until the assay was performed.

Assay of plasma oxytetracycline

Determination of oxytetracycline concentrations in the plasma was done by competitive enzyme-linked immunoabsorbent assay (ELISA) with a commercial quantitative ELISA kit for oxytetracycline (Shenzhen Lvshiyuan Biotech Co, Ltd, Guangdong, China). The sensitivity of the kit was 0.40 ng/mL, with a recovery rate of 90% ± 15%. The lower limit of quantification for the kit was 6.0 ng/mL, whereas the correlation coefficient for absorption percentage of the standard solutions and the semilogarithm values of the oxytetracycline standard solutions was –0.9327 within the concentration range of 4–108 ng/mL. Plasma concentrations at different time points were calculated from the calibration plot.

Pharmacokinetic parameters analysis

The maximum plasma concentrations (C_{max}) and time to C_{max} (T_{max}) were obtained from the plasma concentration versus time data. Individual 24-hour pharmacokinetic parameters were determined with Kinetica pharmacokinetics software (Thermo Scientific, Waltham, MA, USA). The areas under the concentration time curve (AUC) were calculated by the linear trapezoidal rule with extrapolation to infinity ($AUC0_{-24h}$ and $AUC_{0-\infty}$).

Statistical analysis

Data are presented as mean (± SEM). They were evaluated with SPSS Statistical Data Editor Version 20.0 software (IBM, Armonk, NY, USA). Data were analyzed by 1-way analysis of variance by Turkey post hoc test, and results were considered significant at $P < .05$.

Results

A composite mean concentration-time curve from 0.25 to 120 hours after treatment and the corresponding pharmacokinetic parameters are presented in Figure 1 and Table 1, respectively.

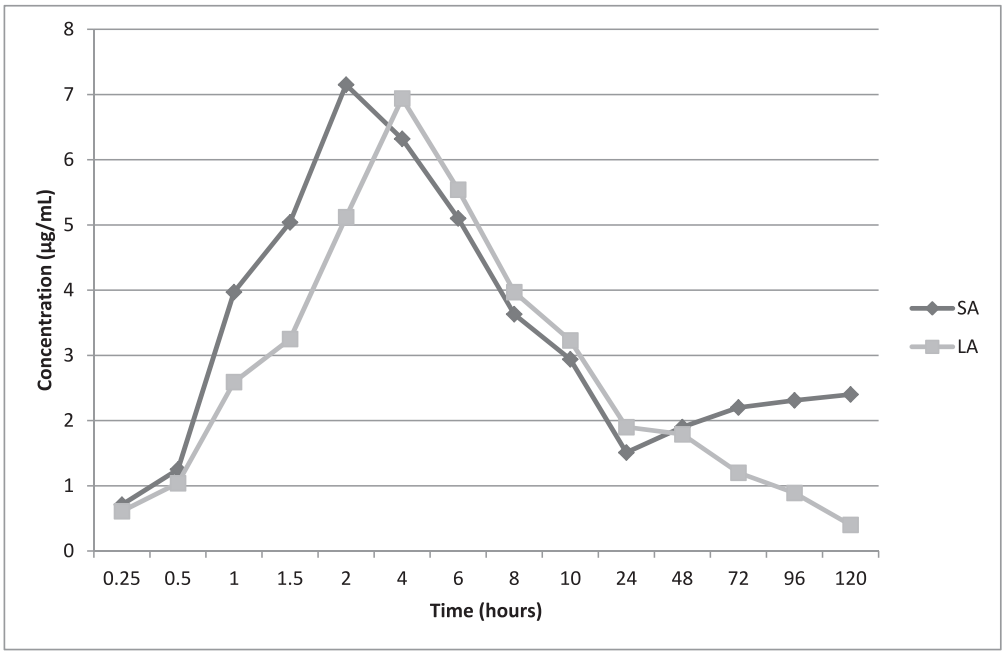

Figure 1. Plasma concentration versus time profiles of oxytetracycline in broiler chickens after intramuscular treatment with short-acting (SA) or long-acting (LA) formulation.

Plasma concentrations of oxytetracycline were significantly higher ($P < .05$) in chickens treated with the short-acting formulation when compared with those treated with long-acting formulation during the absorption phase. Conversely, there was no significant difference ($P > .05$) between the 2 formulations during the elimination phase, however plasma concentration was higher in chickens treated with long-acting formulation. During the 24-hour interval sampling, plasma levels of oxytetracycline were significantly lower ($P < .05$) in chickens administered the long-acting formulation compared with those on short-acting formulation. The mean C_{max} for short-acting and long-acting

Table 1. Pharmacokinetic parameters of oxytetracycline in chickens after intramuscular administration of short- or long-acting formulation.

	Dosage formulation[a]		
Pharmacokinetic parameter[b]	Short acting[c]	Long acting[d]	*P* value
C_{max}, µg/mL	7.15 ± 0.26	6.94 ± 0.05	.43
T_{max}, h	2.0 ± 0.0	4.0 ± 0.0	<.001
α, h^{-1}	3.22 ± 0.13	1.58 ± 0.02	<.001
AUC_{0-24h}, mg/L per h	76.94 ± 4.26	80.78 ± 4.53	.55
$AUC_{0-\infty}$, mg/L per h	108.2 ± 26.1	130.5 ± 35.2	.29
β, h^{-1}	0.06 ± 0.007	0.04 ± 0.009	.27
$T_{1/2}$ β, h	13.56 ± 1.7	17.00 ± 2.39	.27
MRT, h	18.70 ± 2.39	24.40 ± 3.32	.20
Vd, L/kg	3.42 ± 0.26	1.86 ± 0.16	.001*
Cl, L/h	0.19 ± 0.02	0.84 ± 0.02	.003*

Abbreviations: C_{max} indicates maximum plasma concentration; T_{max}, time to C_{max}; α, mean absorption rate constant; AUC, area under the curve; β, elimination rate constant; $T_{1/2}$, elimination half life; MRT, mean residence time; Vd, volume of distribution; Cl, clearance.
[a] Data are presented as mean ± SEM (n = 7).
[b] For a 24-hour sample.
[c] 10 mg/kg IM q24h ×4d.
[d] 20 mg/kg IM once.
* Difference between parameters is statistically significant at $P < .05$.

oxytetracycline was 7.07 and 6.94 μg/mL, respectively, with a corresponding T_{max} of 2 hours and 4 hours. The mean plasma concentration of oxytetracycline during the study period was above 0.5 μg/mL, except for the group treated with the long-acting formulation, where the mean value was 0.40 ± 0.08 μg/mL at 120 hours (5 days after initial single treatment).

The mean absorption rate constant (α) of oxytetracycline was significantly higher ($P < .05$) in chickens administered the short-acting formulation than it was in those treated with the long-acting formulation. No significant difference ($P > .05$) was observed in the mean pharmacokinetic parameters, such as $AUC_{0–24h}$, $AUC_{0–\infty}$, elimination rate constant (β), elimination half life ($T_{1/2}$), and mean residence time. However, values obtained in the group treated with long-acting oxytetracycline formulation were relatively higher, except for mean elimination rate constant (β), where the reverse was observed.

Discussion

For time-dependent antimicrobial agents, efficacy is maximized when plasma drug concentration is above the minimum inhibiton (MIC_{90}) of the infecting bacterial organism for ≥50% of the dosing interval.[4] A reference interval for oxytetracycline MIC of 0.1–1.0 μg/mL against common susceptible bacterial organisms has been reported.[5,6] Target therapeutic tetracycline plasma concentration of ≥1 μg/mL for *Chlamydia* species in Japanese quail (*Coturnix japonica*) has been recommended.[7] Clinical studies have also reported that lower tetracycline plasma concentrations (<1 μg/mL) may be effective against susceptible *Chlamydia* species in parrots (*Amazona viridigenalis*).[8] In this study, a therapeutic plasma concentration of oxytetracycline was maintained in animals treated with both short- and long-acting formulations up to 100% of the respective recommended dosing intervals of 24 and 72 hours. This satisfies the requirements of ≥50% and 40%–50% for therapeutic efficacy of the time-dependent antimicrobial agents.[4,9] This subsequently supports the manufacturer's recommended dosages for oxytetracycline of 10 mg/kg IM q24h for 3–4 consecutive days and 20 mg/kg IM once to be repeated after 3 days (72 hours) if necessary for short- and long-acting formulations, respectively.

The area under the plasma concentration versus time curve ($AUC_{0–24h}$) depicts the level of exposure of the body to an administered and absorbed drug. Our result shows that the level of exposure to oxytetracycline in chickens treated with either the short- or long-acting formulation at recommended therapeutic dosages was comparable. This level was higher than that found in an earlier report in hens after oral treatment with a short-acting formulation at a dose of 10 mg/kg and in Japanese quail after an intravenous dose of 20 mg/kg.[7,10]

The rate of oxytetracycline absorption was significantly slower in chickens treated with the long-acting formulation compared with those administered short-acting formulation. However, the therapeutic plasma concentration was maintained within the dosing interval of 72 hours. This confirms the fact that long-acting oxytetracycline is formulated to act longer by reducing the rate of release and absorption from the site of administration. Considering that both formulations provided therapeutic plasma concentrations of oxytetracycline throughout the recommended period within their respective dosing intervals, we suggest that both are effective in treating nonresistant and susceptible bacterial infections in chickens. However, the high cost of treatment in terms of total amount of the active drug used, frequency of visits to the farm, and stress as well as mortalities from handling of animals for treatment are the limitations of long-acting formulation. Injection site reactions, including swelling, necrosis, and yellow discoloration, were seen almost inevitably after extra-venous routes such as intramolecular and subcutaneous treatment with oxytetracycline.[4,11] Consequently, multiple daily IM injections required for the short-acting formulation might render the carcass objectionable to the consumers, hence causing a loss in economic value. In addition, the 21-day withdrawal period indicated for short-acting formulation will be extended following multiple treatments.

Both short- and long-acting formulations of oxytetracycline maintained therapeutic concentrations for the required period after treating broiler chicken with the respective required dosages. However, considering the high cost and public health significance due to extended withdrawal period as well as damage to edible tissues after multiple injections of the short-acting formulation, we recommend the use of the long-acting formulation of oxytetracycline in broiler chickens that are primarily raised for meat.

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