Published in final edited form as:

J Vet Pharmacol Ther. 2014 December ; 37(6): 607–610. doi:10.1111/jvp.12144.

Pharmacokinetics of oral chlortetracycline in nonpregnant adult ewes

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Abstract

The objectives of this study were to determine plasma concentrations and pharmacokinetic parameters of feed-grade chlortetracycline (CTC) in sheep after oral administration of 80 or 500 mg/head daily, divided into two equal doses given at 12-h intervals for 8 days. These are the approved, and commonly used but unapproved, feed additive doses, respectively, in the United States for the prevention of ovine infectious abortion.

Blood samples were collected just prior to dosing at 0, 12, 24, 72, 96, and 192 h, as well as 4, 8, 12, 24, and 36 h after the last dose, and noncompartmental pharmacokinetic analysis was performed to estimate elimination half-life and area under the plasma concentration–time curve (AUC).

Mean observed maximum CTC concentrations ($C_{\max}$) were 20.0 ng/mL (80 mg dose) and 101 ng/mL (500 mg dose). Mean apparent elimination half-life was 18 h (80 mg dose) and 20 h (500 mg dose).

Although published data do not exist to estimate plasma CTC concentrations necessary for the prevention of ovine infectious abortion, concentrations reached in our study suggest that either the FDA-approved and FDA-unapproved dosages are not high enough or that the pharmacodynamic parameter relating preventive dose to pathogen minimum inhibitory concentrations is yet to be determined.

INTRODUCTION

Infectious ovine abortions are responsible for significant economic losses due to abortion storms within the sheep industry (Anonymous, 2011). Infections with *Campylobacter* spp. are a common cause of ovine abortion throughout the world and have been reported as the most common cause of infectious ovine abortion in the United States (Sahin *et al.*, 2008). Susceptible ewes, upon exposure, develop placentitis and abortion, usually in the last trimester of pregnancy (Edmondson *et al.*, 2012). Once abortions within the flock begin, other ewes in the flock may be exposed to very high levels of *Campylobacter* through contact with aborted fetuses, placentas, and uterine fluids (Edmondson *et al.*, 2012). Further, many *Campylobacter* spp., most importantly *Campylobacter jejuni*, are zoonotic diseases; therefore, exposure is hazardous for humans. Intervention measures that are useful for treating and preventing ovine abortions from campylobacteriosis are paramount for animal and human health.

Traditional recommendations for treating and preventing *Campylobacter*-associated infection and abortion in flocks have focused on administering chlortetracycline (CTC) or tetracycline in the feed throughout the last trimester. The FDA-approved dose in the United States for this use of chlortetracycline is 80 mg/head/day (Anonymous, 2013). A large percentage of *Campylobacter* isolates responsible for ovine abortion in the United States are considered resistant to tetracyclines (Sahin *et al.*, 2008), and there are anecdotal reports of a lack of efficacy. Resistance of *Campylobacter* isolates to tetracyclines and the reported treatment failures suggest that the currently approved dose does not provide therapeutic drug concentrations in the target tissues, and it is possible that low concentrations of drug have led to the development of resistance. Feeding of an extra-label dose of chlortetracycline of up to 500 mg/head/day may result in increased efficacy of managing sheep flocks in the last trimester to prevent abortion outbreaks from campylobacteriosis (Menzies, 2011). To date, no studies have examined concentrations of chlortetracycline achieved in nonpregnant or pregnant sheep following the oral administration of feed grade chlortetracycline at either 80 or 500 mg/head/day. Further, the extra-label use of drugs in feed is illegal in the United States according to the Animal Medicinal Drug Use Clarification Act (Anonymous, 1996). Therefore, before label approval is sought, knowledge of CTC concentrations following administration of this extra-label dose is desirable.

The objectives of our study were to determine the plasma concentrations and pharmacokinetic parameters of chlortetracycline following oral administration to adult sheep for 8 days. Inferences as to the potential efficacy or lack of efficacy of the practice of feeding pregnant sheep chlortetracycline during the last trimester to prevent infectious ovine abortion from *Campylobacter* spp. might be possible from these data.

The experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University. Twelve healthy, open, adult ewes (2–5 years of age; 56–104 kg) were housed individually indoors, but within sight of flock-mates at all times, and fed free choice hay and water and a commercially prepared ration throughout the study. Ewes were randomly assigned to one of two treatment groups. Six ewes received 80 mg/head CTC orally for 8 days in divided doses (40 mg/head, 12 h apart),

and six ewes received 500 mg/head CTC orally for 8 days in divided doses (250 mg/head, 12 h apart). Doses were prepared by mixing CTC crumbles (Aureomycin 4 g crumbles, Nutra Blend Company, Neosho, MO, USA) with clear corn syrup in a 20 cc syringe. The end of the syringe was removed in order to facilitate oral administration.

Blood samples were collected just prior to dosing at 0, 12, 24, 72, 96, and 192 h, as well as 4, 8, 12, 24 and 36 h after the last dose was administered. All blood samples were collected by jugular venipuncture into a tube containing lithium heparin, immediately separated via centrifuge, and stored at -80°C until analysis.

Concentrations of CTC in plasma were analyzed by high-performance liquid chromatography as previously described (Reinbold *et al.*, 2010). More specifically, plasma samples, plasma spikes, and plasma QC samples, 0.200 mL, were treated with 1 M trichloroacetic acid (100 μL) after addition of the internal standard demeclocycline (10 μL of 10 ng/ μL). The samples were vortexed for 5 sec and centrifuged for 20 min at 2000 *g* to sediment the precipitate. A portion of supernatant, 150 μL , was transferred to an injection vial fitted with a glass insert containing 60 μL of 1.9% ammonium hydroxide in 25% aqueous acetonitrile. The injection volume was set to 20 μL . The mobile phases consisted of A: 0.1% formic acid in water and B: 0.1% formic acid in methanol at a flow rate of 0.25 mL/min. The mobile phase began at 15% B with a linear gradient to 95% B at 5 min, which was maintained for 1.5 min, followed by re-equilibration to 15% B. Separation was achieved with a C18 column (Intersil ODS4, 75×2.1 mm, 3 μm particles) maintained at 45°C . Demeclocycline eluted at 4.3 min and chlortetracycline at 4.7 min. Three SRM transitions were monitored for both chlortetracycline and demeclocycline. The quantifying ions for chlortetracycline were 154, 444, and 462 *m/z* and 154, 289, and 448 *m/z* for demeclocycline. Calibration spikes and QC samples were prepared in blank sheep plasma. Sequences consisting of plasma blanks, calibration spikes, QC samples, and sheep plasma samples were batch-processed with a processing method developed in the Xcalibur software (Thermo Scientific, San Jose, CA, USA). The processing method automatically identified and integrated each peak in each sample and calculated the calibration curve based on a weighted (1/*X*) linear fit. Plasma concentrations of chlortetracycline in unknown samples were calculated by the Xcalibur software based on the calibration curve. Results were then viewed in the Quan Browser portion of the Xcalibur software. Ten calibration spikes ranging from 1 to 1000 ng/mL were used to establish the calibration which exhibited a correlation coefficient exceeding 0.995. Duplicate QC samples were prepared with each set of samples at three concentration levels of 2.5, 25, and 250 ng/mL. The limit of detection (LOD) of the method was <1 ng/mL, and the LOQ was 1 ng/mL.

Noncompartmental analysis was performed to estimate various parameters (Phoenix WinNonlin, Certara, 6.3.0.395 Pharsight, St. Louis, MO, USA). Plasma concentrations of CTC in the 500 mg treatment group appear proportionally higher than in the 80 mg treatment group (Fig. 1). Large standard deviations of plasma concentrations suggest underlying mechanisms related to differences in absorption, pharmacogenetic differences, or other animal characteristics that add variability to either bioavailability or drug disposition. However, our study design, logistics, and drug availability did not permit further investigation of factors associated with variance.

Mean apparent elimination half-life was 18 h (80 mg daily dose) and 20 h (500 mg daily dose) (see Table 1). Even though there were not enough sampling times to determine whether steady-state was reached, steady-state is expected at 4–5 half-lives, or approximately 90–100 h after initial dosing. Visual inspection of the time–concentration data suggests steady-state had been reached by the 5th or 6th day of dosing.

Although published data do not exist to estimate effective plasma CTC concentrations for the prevention of ovine abortion from *Campylobacter* spp., concentrations reported here are substantially below the minimum inhibitory concentration (MIC) of tetracyclines reported in the literature for most *C. jejuni* isolates collected from livestock and isolates associated with abortion outbreaks (Sahin *et al.*, 2008; Oporto *et al.*, 2009; Scott *et al.*, 2012). Subinhibitory levels of tetracyclines have been shown to have inhibitory effects on bacteria or bacterial processes *in vitro* or in the environment (although not in *Campylobacter* spp.), which may explain some of the clinical effects historically reported from the successful use of tetracyclines in the feed for ovine infectious abortion (Vosbeck *et al.*, 1982; Comby *et al.*, 1989; Tetz & Norman, 1994; Knapp *et al.*, 2008; Tanabe *et al.*, 2013). On the other hand, low levels of tetracyclines have been reported to enhance resistance gene acquisition in gastrointestinal pathogens (Rashtchian *et al.*, 1982; Brewer *et al.*, 2013). The AUC_{0–24}:MIC is another pharmacodynamic parameter reported to be useful in predicting efficacy for some tetracyclines (Turnidge & Paterson, 2007). In this study, the mean AUC_{0–24} was 1801 ng/mL after 250 mg/head twice a day (500 mg daily dose), so the ratio for isolates with a reported MIC₅₀ of 64 mcg/mL would be 0.03. The low values in our study of time > MIC and AUC_{0–24} suggest that either the FDA-approved and commonly used dosages are not high enough or that the pharmacodynamic parameter relating preventive dose to pathogen MIC is not time above MIC or AUC_{0–24}.

ACKNOWLEDGMENTS

This study was funded by the Department of Veterinary Physiology and Pharmacology, Texas A&M University, College of Veterinary Medicine, College Station, TX and NIH AA18166-2 (SW). Special thanks to Dillon Johnson, Sarah Baker, and Lance Wheeler for their assistance.

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Fig. 1. Plasma concentrations (ng/mL) of chlortetracycline over time (in hours) in open, adult ewes after 40 mg/head q12 h or 250 mg/head q12 h via oral dosing for 8 days. Error bars are standard deviations.

Table 1

Pharmacokinetic parameters from noncompartmental analysis after the last dose of chlortetracycline administered orally twice a day for 8 days. Nominal dose was 40 mg or 250 mg/head twice a day; final dose was calculated from ewe body weights

Animal No.	40 mg/head q12 h							250 mg/head q12 h								
	520	2042	2047	2065	10 947	10 963	Mean	SD	83	929	2021	2027	2064	2076	Mean	SD
Dose (mg/kg)	0.4	0.5	0.5	0.7	0.7	0.7	0.6	0.1	3.7	3	3.5	3.7	3.4	3.9	3.5	0.3
T_{max} (h)	4	0	4	4	0	0	2	2	4	8	8	4	4	0	5	3
C_{max} (ng/mL)	9.5	6.3	19.6	25.4	40.9	15.4	20	13	157	83	118	92	93	60	101	33
λ_z (/h)	0.042	0.030	0.061	0.044	0.049	0.024	0.042	0.013	0.047	0.030	0.035	0.059	0.032	0.023	0.038	0.013
$t_{1/2\lambda_z}$ (h)	16.7	22.9	11.4	15.8	14.1	29.2	18.4	6.5	14.9	22.9	19.7	11.7	21.7	30.5	20.2	6.6
AUC_{last} (ng·h/mL)	261.4	135	426.8	479.8	721.4	365.6	398	200	3650	2029	3116	1290	2078	1537	2283	918
AUC_{INF_obs} (ng·h/mL)	346	201	489	658	860	694	541	243	4539	2947	4353	1469	3048	2725	3180	1134
AUC% extrapol	24.3	32.9	12.8	27.1	16.1	47.3	27	12.4	19.6	31.2	28.4	12.2	31.8	43.6	27.8	10.8
$AUMC_{last}$ (ng ² ·h/mL)	3906.4	1960	5569.6	5921.6	9123.2	5747.2	5371	2381	49 369	29 784	46 554	14 600	30 811	23 565	32 447	13 361
$AUMC_{INF_obs}$ (ng ² ·h/mL)	8957	6529	8857	16 394	16 947	31 361	14 841	9157	100 427	93 152	126 214	24 080	96 014	118 540	93 071	36 248
MRT_{last} (h)	14.9	14.5	13.0	12.3	12.6	15.7	13.9	1.4	13.5	14.7	14.9	11.3	14.8	15.3	14.1	1.5
MRT_{INF_obs} (h)	25.9	32.5	18.1	24.9	19.7	45.2	28	10	22.1	31.6	29.0	16.4	31.5	43.5	29	9
AUC_{0-24} (ng·h/mL)	202.6	103.2	355.4	405.4	595.4	272	322	172	2967	1564	2400	1117	1604	1154	1801	735

SD, standard deviation; C_{max} , observed maximal concentration; T_{max} , time at observed maximal concentration; $t_{1/2\lambda_z}$, apparent elimination half-life estimated from the terminal time-concentration curve; λ_z , first-order rate constant associated with the terminal portion of the time-concentration curve; AUC_{last} , area under the curve from time zero to the last sample time; AUC_{INF_obs} , area under the curve from time zero extrapolated to infinity; $AUC\%$ extrapol, percent AUC extrapolated from last sample time to infinity; $AUMC_{last}$, area under the moment curve from time zero to last sample time; $AUMC_{INF_obs}$, area under the moment curve from time zero extrapolated to infinity; MRT_{last} , mean residence time based on time zero to last sample time; MRT_{INF_obs} , mean residence time based on time zero extrapolated to infinity; AUC_{0-24} , area under the curve for the first 24-h dosing interval.