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Effect of Diminazine Aceturate on the Pharmacokinetic of a Long Acting Oxytetracycline Formulation in Lactating Goats

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SUMMARY

Oxytetracycline (OTC) and diminazene aceturate are commonly administered to diseased ruminants with mixed bacterial and protozoal infections. We were therefore interested in characterizing the pharmacokinetics of a new long acting OTC formulation after IV or IM administration, and whether concurrent administration of diminazene altered the pharmacokinetics. Ten clinically healthy lactating female Baladi goats were used in a sequential order. Goats received the treatments in sequential order with a 2 week wash out period between each study: 1) a single dose of OTC (30 mg/kg BW) by IV or IM injection in non-treated and diminazine aceturate pre-treated goats (3.5 mg/kg BW) 2 hours before OTC treatment. Blood, milk and urine samples were collected periodically and OTC concentration was assayed using a microbiological method. The extent of protein binding in serum and milk was determined using an in vitro ultrafiltration method and assayed using the same method as serum Pharmacokinetic analysis indicated that serum OTC concentrations after IV administration could be fit to a two-compartment model, and that pre-treatment with diminazene aceturate increased serum OTC concentrations. Following IV injection ($t_{0.5}$ β) was 25.9 ± 5.1 and 24.5 ± 2.7 hours, and (Vd_{area}) was 22.0 ± 0.8 and 23.7 ± 0.4 $L.kg^{-1}$, in non-treated and diminazine pre-treated goats, respectively. The maximum OTC concentration after IM injection (1.25 ± 0.02 $\mu g.ml^{-1}$ and 1.39 ± 0.04 $\mu g.ml^{-1}$) was obtained at 1.8 ± 0.3 hours and 2.4 ± 0.4 hours in non-treated and diminazine pre-treated goats, respectively. Moreover, effective milk concentrations were detected for 24 to 48 h, and effective urine concentrations were

detected for 96 to 120 h after IM injection. The LA-OTC formulation was moderately bound to goat serum protein (46.0 ± 3.2 % for OTC alone and 40.0 ± 2.3 % for OTC + diminazine). The binding of the LA-OTC formulation was lower in milk (29.3 ± 3.6 %) than plasma.

We conclude that concurrent administration of LA-OTC and diminazine aceturate alters the serum concentration-time profile and pharmacokinetics of a new long acting OTC formulation and could therefore potentially alter treatment efficacy.

Keywords: oxytetracycline, diminazene aceturate, protein binding, bioavailability

INTRODUCTION

Oxytetracycline (OTC) is a broad spectrum bacteriostatic antimicrobial agent that is widely used in veterinary medicine for the treatment of respiratory and gastrointestinal infectious diseases. Oxytetracycline is active against aerobic Gram-positive and Gram-negative bacteria, rickettsia, mycoplasma, and chlamydia (Baxter & McKellar, 1995; Riviere and Spoo, 1995). Oxytetracycline is moderately bound to plasma proteins in goats (40-50%, Varma and Paul, 1983; 67%, Anika et al., 1986), is minimally metabolized in the body, and is primarily cleared through the urinary system (Aronson, 1980). Protein binding of OTC appears to be predominantly to hydrophobic pockets at or near site I within sub-domain IIA of the albumin molecule, and ionic binding of OTC to albumin appears to be of minor importance (Khan et al., 2002). Long-acting oxytetracycline (LA-OTC) formulations are the treatment of choice for some acute and chronic infectious diseases of ruminants (Cornwell, 1980), and including cattle (Xia et al., 1983; Mevius et al., 1986; Fourtillan et al., 1989; Meijer et al., 1993), sheep (Nouws et al., 1985), goats (Escudero et al., 1994; Escudero et al., 1996) and sheep and calves (Craigmill et al., 2000) as well as control and eradication of Bluetongue virus 8 against five in cattle and sheep (Fofana, et al., 2009). The first aim of the study reported here was to characterize the pharmacokinetics of a new LA-OTC formulation following IV or IM administration in lactating goats. The studied formulation contains a higher OTC concentration (30%) than other formulations (10-20% OTC) and dimethylacetamide as a solvent which provides a low viscous solution that is easy to inject. We selected lactating goats as the ruminant model to study because of their economic importance in Egypt, the ease in obtaining milk samples, and because of their cost and ready availability relative to cattle. African trypanosomiasis affected the lives of people living in sub-Saharan African at all times, African

trypanosomiasis is an infectious disease of humans and animals of similar aetiology and epidemiology. The causative agents of the disease are protozoan parasites of the genus *Trypanosoma* that live and multiply extracellularly in blood and tissue fluids of their mammalian hosts and are transmitted by the bite of infected tsetse flies (*Glossina* sp.), Molyneux et al., (1996). Diminazene aceturate is highly effective drug against trypanosomiasis and babesiasis (Klatt and Hajdu, 1976), and is commonly used to treat trypanosomiasis in cattle, sheep and goats (Silayo, et al., 1992). Diminazene is a dicationic amidine that is highly water soluble and moderately bound to plasma proteins (El-Banna et al, 1998; Mamman et al, 1993) with 60-90% and 65-85% being bound to plasma proteins in goats (Aliu et al., 1984) and sheep (Aliu and Odegaard, 1985), respectively. Long acting OTC formulations and diminazene are administered concurrently to some diseased ruminants with mixed bacterial and protozoal infections, which considered as one of the common mixed infections in the Egyptian animals. Combined administration of LA-OTC and diminazene is not suspected to alter the efficacy of diminazene treatment because administration of tetracycline had no effect on the trypanocidal efficacy of diminazine in mice infected with *Trepanosma bruci* (Jennings, 1987). However, concurrent administration of LA-OTC and diminazene may alter the treatment efficacy of LA-OTC because oxytetracycline is moderately bound to plasma proteins in the goat (Varma and Paul, 1983; Anika et al., 1986). Changes in plasma protein binding percent due to displacement by another drug can result in a drug interaction because of altered pharmacokinetics (Rolan, 1993), such as a change in the volume of distribution and maximal plasma concentration. Support for an effect of diminazene on OTC pharmacokinetics is provided by a study in cattle whereby concurrent administration of rolitetracycline and diminazene caused a marked decrease in the elimination half life of diminazene (Klatt and Hajdu, 1976) which was attributed to displacement of diminazene from plasma and other binding sites by rolitetracycline (Aliu and Odegaard, 1985). Accordingly, the second aim of the study reported here was to determine whether intramuscular administration of diminazene altered the pharmacokinetic parameters for IV or IM administration of a new LA-OTC formulation in lactating goats.

MATERIAL AND METHODS

Drugs:

A long acting OTC hydrochloride formulation (Alamycine®; Norbrook,

England), structural formula 2Naphtha cenecarboxamide,[4s-(4aa,5a,5aa,6b,12aa)]-4-(dimethyl amino)., 4,4a,5,5 a,6,11,12a-octahydro-3,5,6,10,12,12a,hexahydroxy-6-methyl-1,11-dioxo dehydrate, was obtained as a yellow solution in vials of 100 ml (300 mg of OTC base/ml in dimethylacetamide solvent). Diminazene aceturate (Norotryp®; Norbrook, England), structural formula 4,4'-diamidinodiazoaminobenzene diaceturate tetrahydrate, was obtained as a water soluble yellow powder in cachets of 2.36 g granules equivalent to 1.05 g active ingredient.

Animals:

Ten clinically healthy lactating female Baladi goats (20 kg body weight; 24 to 32 months of age) were obtained from local sources. Goats were housed in a clean stall and fed alfalfa ad libitum and a concentrate mixture composed of 91% maize bran, 8% hay and 1% common salt (NaCl). The concentrate mixture contained 12.7% crude protein and 19.7 MJ GE (approximately 13 MJ ME)/kg DM and was graciously prepared by Department of Nutrition in the Faculty of Veterinary Medicine at Cairo University. Water was offered ad-libitum. Goats were observed for a month before the start of the experiment to ensure adequate clearance of any previously ingested drugs or chemical substances that could potentially alter plasma protein binding or metabolism.

Experimental procedure:

A sequential order was carried out in this experiment. A 22 gauge 5 cm catheter was aseptically placed in the right jugular vein and secured to the neck at least 30 minutes before treatments were administered. A 16-F Foley catheter (Folatex norta, B.D.F. Belers Dort A.G. Hamburg 16 Fl/ch 4.7mm 5.15c.c/ml) was placed into the bladder and the bladder emptied of urine by aspiration immediately before drug injection.

Five goats received the following 2 treatments in sequential order with a 2 week wash out period between each study: a single dose of OTC (30 mg/kg BW;) by IV injection; a single dose of OTC (30 mg/kg BW) by IM injection (Slight transient swelling for less than 1 hour was observed at site of injection) . The remaining 5 goats received the following 2 treatment in sequential order with a 2 week wash out period between each study: IM administration of diminazene (3.5 mg/kg BW) 2 hours before IV or IM injection of LA-OTC (30 mg/kg BW). Intravenous injection of OTC was into the left jugular vein and was completed within 60 seconds. Intramuscular injections of OTC and diminazene were into the cervical musculature. The dose of diminazene was selected according to Klatt and Hajdu, (1976); Aliu and Odegaard, (1985) and Mamman et al., (1993).

Sampling and Analysis of Biological Fluids:

Blood samples (10 ml) were collected via the catheter in the right jugular vein immediately before treatment, and at intervals of 5, 10, 15 and 30 minutes, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 hours after OTC administration. Blood samples were placed in plain glass tubes, allowed to clot at room temperature, and centrifuged at 1000 g for 15 minutes. Serum was harvested and kept at -٢٠°C until analyzed.

Milk samples were obtained by hand milking all available milk in the udder immediately before OTC was administered and at 1, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 hours post injection. Milk samples were collected into clean sterile glass tubes and the pH determined immediately after collection of the milk sample using a pH meter (Oakton® Acorn™ Series). Milk samples were centrifuged at 1000 g for 15 minutes and the fat free portion harvested and kept at -20°C until analyzed.

Urine samples were collected via the catheter in the bladder at 1, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 hours post-injection of OTC. The volume and pH of each urine sample were measured immediately after collection. Urine samples were stored at -٢٠°C until analyzed. At the end of each study the urinary bladder was irrigated with 20 to 30 ml potassium permanganate solution (1:5000) as an antiseptic agent.

Oxytetracycline concentration in serum, milk and urine was determined using a standard microbiological assay method (Arret *et al.*, 1971; Walton, 1983) based on *Bacillus subtilis* (ATCC 6633) which was generously provided by the Microbiology department, the Faculty of Veterinary Medicine, Cairo University. The OTC concentration was assayed using 6 well agar plate for each serum, milk and urine sample. Three wells on each plate were filled with the reference concentration of OTC (1.0 µg/ml) diluted in the appropriate diluent (antibiotic-negaive pooled serum and milk, phosphate buffer for urine) and the other three wells were filled with the sample (serum, milk and urine). Plates were incubated at 37°C for 16-18 hours and the diameter of each inhibition zone measured and indexed to the mean diameter of the inhibition zone of the reference concentration. Each assay of standard and unknown was carried out in triplicate. The inhibition zone diameter for the sample were converted into concentrations by using the appropriate standard curve (Pearson correlation coefficients for the 5 point standard curves were: buffer, r = 0.982; serum, r = 0.988; milk, r = 0.901; urine, r = 0.957). The lowest limit of quantification was 0.5 µg/ml and the intraassay coefficient of variation was less than 5%. The

bacteriologic method was validated using a spectrophotometric method as described by (Snell and Snell 1971) using serum samples from 5 goats obtained at 1 hour after IM injection of OTC. The plasma OTC concentration obtained using the microbiologic method (1.25 ± 0.02 μg/ml) was similar to that obtained using the spectrophotometric method (1.27 ± 0.02 μg/ml).

The extent of protein binding in serum and milk was determined using an in vitro ultrafiltration method which is based on the diffusion of the free (unbound) OTC into the agar medium (Lorian, 1980). Jugular venous blood samples were placed in plain glass tubes, allowed to clot at room temperature, and centrifuged at 1000 g for 15 minutes. Serum was spiked with OTC alone at 1.00 and 1.56 μg/ml. Separate serum samples were spiked with diminazine at 1.50 and 3.00 μg/ml and two hours later the samples were spiked with oxytetracycline at 1.00 and 1.56 μg/ml. Three replicates of the spiked samples were kept for 2 hours to permit OTC and diminazine to bind with serum proteins. The serum and milk OTC concentrations were assayed using the microbiological assay method described previously as the difference in zone inhibition between centrifuged and uncentrifuged samples. Protein binding in milk was assayed using the same method as serum.

Pharmacokinetic analysis:

Compartmental pharmacokinetic parameters were calculated by applying a 2 compartment open model to the OTC concentration-time relationship after IV administration as described (Baggot, 1978). A 2 compartment model was selected to assist in comparison to previous studies in different animals that utilized a 2 compartment model following IV injection of conventional or long-acting OTC formulations in goats (Escudero et al., 1994), cattle (Toutain and Raynaud, 1983), sheep (Ponferrada, 1987), horses (Horspool and Mc-Kellar, 1990), rabbits (McElory et al., 1987) and hens (Moreno et al., 1996). The following biexponential equation, which assumes first order kinetics for elimination from the central compartment was therefore applied: $C(t) = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t}$, where α and β are hybrid constants. A 1 compartment open model was applied to the OTC concentration-time relationship after IM administration as described (Baggot, 1978 and Riviere, 1999). A lag time for absorption was not discernable in our IM data and was therefore considered to equal zero. The peak concentration (C_{max}) and time to reach peak concentration (t_{max}) following IM administration of LA-OTC were calculated as described (Yamaoka *et al.* (1978).

The area under the concentration-time curve (AUC) and the area under the first

moment concentration-time curve (AUMC) were calculated from compartmental values as: $AUC = A/\alpha + B/\beta$, and $AUMC = A/\alpha^2 + B/\beta^2$. The mean residence time (MRT) was calculated using the ratio AUMC/AUC (Toutain and Kortiz, 1997). Bioavailability was calculated as: Bioavailability % = $(AUC_{IM} \times 100)/AUC_{IV}$.

Statistical analysis:

Data were expressed as mean ± SD and a P value < 0.05 was considered significant. Repeated measures analysis of variance was used to determine the effects of treatment and time and treatment-time interaction using PROC MIXED (SAS 9.1; SAS Institute, Cary NC).

RESULTS

Mean serum, milk and urine concentrations of OTC following a single IV or IM injection of a new LA-OTC formulation in non-treated and diminazene aceturate pre-treated lactating goats indicated that the OTC concentrations were higher in diminazene pre-treated goats than in non-treated goats (Figures 1 and 2; Tables 1 and 2).

Pharmacokinetic modeling of the serum OTC concentration-time data following IV injection of LA-OTC indicated that the volume of distribution was greater and the rate of distribution to the peripheral compartment was slower in diminazene aceturate pre-treated goats than in non-treated goats (Table 3). Mean residence time was also longer in diminazene aceturate pre-treated goats. However, total body clearance, the rate of elimination, and the elimination half-time were similar in non-treated and pre-treated goats.

Following intramuscular injection of LA-OTC, the maximum calculated serum concentration (C_{max}) was higher and t_{max} was longer in diminazene aceturate pre-treated goats than in non-treated goats, respectively (Figure 2, Table 4). The rate of absorption was slower and the absorption half time was correspondingly longer in diminazene aceturate pre-treated goats than in non-treated goats. However, the rate of elimination and the elimination half-time were similar in non-treated and pre-treated goats. Bioavailability was 88.1 ± 1.7 and 99.6 ± 15.0% following IM injection of LA-OTC in non-treated and diminazine pre-treated goats, respectively. The MRT following intramuscular injection was 42.5 ± 8.1 and 35.2 ± 3.9 hours for non-treated and diminazine pre- treated goats, respectively.

The OTC concentration in milk was maintained above 0.50 μg/ml for approximately 48 hours in goats administered intramuscular LA-OTC, with or without diminazene aceturate pre-treatment (Table 2). The OTC concentration in urine was maintained above 0.50 μg/ml for at least 96 hours in goats

administered intramuscular LA-OTC, with or without diminazene aceturate pre-treatment (Table 2).

The degree of plasma protein binding by OTC was significantly decreased (P = 0.039) by pre-adding of diminazene. The LA-OTC formulation was moderately bound to goat serum protein (46.0 ± 3.2 % for OTC alone and 40.0 ± 2.3 % for OTC + diminazine). The binding of the LA-OTC formulation was lower in milk (29.3 ± 3.6 %) than plasma.

Table 1: Mean concentrations (M ± SD) of oxytetracycline (OTC, µg/ml) in milk and urine of lactating goats after IV injection of a LA OTC (30 mg/kg BW) with or without an IM injection of diminazene aceturate (3.5 mg/kg BW).

Time	Milk		Urine	
	OTC	OTC + Diminazine	OTC	OTC + Diminazine
5 min.	ND	ND	ND	ND
10 min.	ND	ND	ND	ND
15 min.	ND	ND	ND	ND
30 min.	ND	ND	ND	ND
1 h.	ND	ND	1.46 ± 0.01	1.50 ± 0.03**
2 h.	0.49 ± 0.01	0.54 ± 0.01***	1.36 ± 0.02	1.40± 0.02*
4 h.	0.66 ± 0.01	0.69 ± 0.02**	1.30 ± 0.03	1.33 ± 0.01*
6 h.	0.71 ± 0.01	0.78 ± 0.02**	1.24 ± 0.01	1.28 ± 0.02*
8 h.	0.75 ± 0.01	0.80 ± 0.02**	1.21 ± 0.03	1.21 ± 0.03
12 h.	0.62 ± 0.02	0.78 ± 0.02***	1.11 ± 0.01	1.27 ± 0.01***
24 h.	0.57 ± 0.03	0.72 ± 0.01***	1.07 ± 0.03	1.19 ± 0.01***
48 h.	0.48 ± 0.01	0.53 ± 0.01***	0.99 ± 0.02	0.96 ± 0.02*
72 h.	ND	0.51 ± 0.02	0.70 ± 0.01	0.96 ± 0.05***
96 h.	ND	ND	0.67 ± 0.07	0.90 ± 0.02
120 h.	ND	ND	0.51 ± 0.01	0.76 ± 0.02

(M ± SD, N=5).
*P ≤ 0.05; **P ≤ 0.01; *** P ≤ 0.001

Table 2: Mean concentrations (M ± SD) of oxytetracycline (OTC, μg/ml) in milk and urine of lactating goats after IM injection of LA OTC (30 mg/kg BW) with or without an IM injection of diminazene aceturate (3.5 mg/kg BW).

Time	Milk		Urine	
	OTC	OTC + Diminazine	OTC	OTC + Diminazine
5 min.	ND	ND	ND	ND
10 min.	ND	ND	ND	ND
15 min.	ND	ND	ND	ND
30 min.	ND	ND	ND	ND
1 h.	.0.69 ± 0.02	0.93 ± 0.02	1.10 ± 0.01	1.10 ± 0.04
2 h.	0.70 ± 0.01	0.76 ± 0.03 **	1.09 ± 0.03	1.15 ± 0.07
4 h.	0.72 ± 0.03	0.87 ± 0.06**	1.06 ± 0.03	1.20 ± 0.09*
6 h.	0.67 ± 0.02	0.68 ± 0.03	1.05 ± 0.04	1.22 ± 0.10*
8 h.	0.63 ± 0.02	0.70 ± 0.02***	1.03 ± 0.03	1.24 ± 0.08**
12 h.	0.61 ± 0.03	0.66 ± 0.01*	1.02 ± 0.04	1.21 ± 0.09
24 h.	0.60 ± 0.04	0.63± 0.01*	1.23 ± 0.06	1.14 ± 0.08
48 h.	0.56 ± 0.02	0.53 ± 0.01*	1.16 ± 0.07	0.91 ± 0.06**
72 h.	ND	ND	1.01 ± 0.03	0.77 ± 0.07**
96 h.	ND	ND	0.75 ± 0.07	0.64 ± 0.03
120 h.	ND	ND	0.80 ± 0.05	0.60 ± 0.04

(M ± SD, N=5).
*P ≤ 0.05; **P ≤ 0.01; *** P ≤ 0.001

Table 3: Pharmacokinetic parameters (M ± SD) of oxytetracycline following intravenous injection of 30 mg/kg BW in lactating goats aloe or pre-treated with diminazene aceturate 3.5 mg/kg BW.

Parameters	Unit	Oxytetracycline alone	Oxytetracycline + Diminazene
CP^0	$\mu g.ml^{-1}$	1.90 ± 0.08	1.95 ± 0.06
A	$\mu g.ml^{-1}$	0.53 ± 0.07	0.67 ± 0.07 *
α	h^{-1}	5.14 ± 1.26	2.58 ± 0.35 **
$t_{0.5(\alpha)}$	min	12.6 ± 5.2	17.6 ± 2.7 *
B	$\mu g.ml^{-1}$	1.37 ± 0.05	1.27 ± 0.02 **
β	h^{-1}	0.029 ± 0.003	0.030 ± 0.002
$t_{0.5(\beta)}$	h	25.9 ± 5.1	24.5 ± 2.7
V_C	$l.kg^{-1}$	15.8 ± 0.5	15.5 ± 0.5
Vd_{ss}	$l.kg^{-1}$	22.3 ± 0.8	24.4 ± 0.5 **
$Vd_{(area)}$	$l.kg^{-1}$	22.0 ± 0.8	23.7 ± 0.4 **
K_{21}	h^{-1}	3.58 ± 0.79	1.70 ± 0.27 **
K_{el}	h^{-1}	0.042 ± 0.006	0.044 ± 0.004
K_{12}	h^{-1}	1.63 ± 0.48	0.95 ± 0.13 *
$Cl_{(B)}$	$l.kg^{-1}.h^{-1}$	0.65 ± 0.10	0.67 ± 0.05
$AUC_{0-\infty}$	$\mu g.ml.h^{-1}$	51.9 ± 19.3	45.6 ± 10.3
MRT	h	42.5 ± 8.1	35.2 ± 3.9*

(M ± SD, N=5). *P ≤ 0.05; **P ≤ 0.01; *** P ≤ 0.001.

*P ≤ 0.05; **P ≤ 0.01; *** P ≤ 0.001.

Table 4: Pharmacokinetic parameters of oxytetracycline following intramuscular injection of 30 mg/kg BW in lactating goats aloe or pre-treated with diminazene aceturate 3.5 mg/kg BW. (M ± SD).

Parameters	Unit	Oxytetracycline alone	Oxytetracycline + Diminazene
A	$\mu g.ml^{-1}$	0.38 ± 0.11	0.40 ± 0.06
$K_{(ab)}$	h^{-1}	2.83 ± 0.54	2.07 ± 0.26 *
$t_{0.5(ab)}$	min	17.0 ± 3.2	21.5 ± 2.9*
B	$\mu g.ml^{-1}$	1.27 ± 0.012	1.38 ± 0.055**
K_{el}	h^{-1}	0.029 ± 0.002	0.027 ± 0.006
$t_{0.5(el)}$	h	24.8 ± 3.1	25.7 ± 2.35
C_{max} Calculated Observed	$\mu g.ml^{-1}$	0.93 ± 0.04 1.25 ± 0.02	1.08 ± 0.43** 1.39 ± 0.04
T_{max}	h	1.80 ± 0.28	2.35 ± 0.39*
$AUC_{0-\infty}$	$\mu g.ml.h^{-1}$	45.3 ± 8.7	45.4 ± 10.3 *
Bioavailability	%	88.1 ± 1.7	99.6 ± 15.0

(M ± SD, N=5). *P ≤ 0.05; **P ≤ 0.01; *** P ≤ 0.001.

* Significant at P ≤ 0.05; ** Significant at P ≤ 0.01

Figure 1: Mean serum concentrations of oxytetracycline (μg/ml) in 5 lactating goats that received an intravenous injection of a long acting oxytetracycline formulation (30 mg/kg BW) with (open circles) or without (filled circles) an intramuscular injection of diminazene aceturate (3.5 mg/kg BW) 2 h previously. Values are mean ± SD.

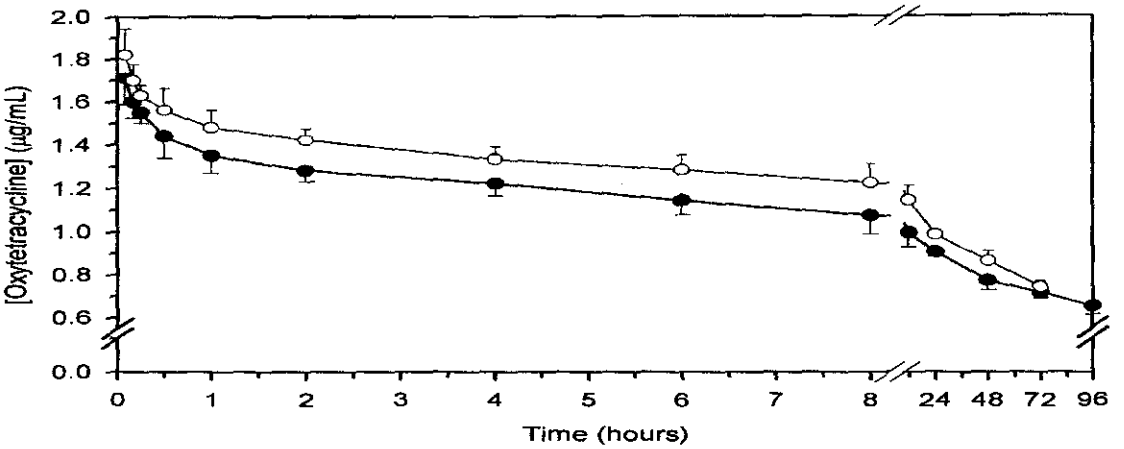
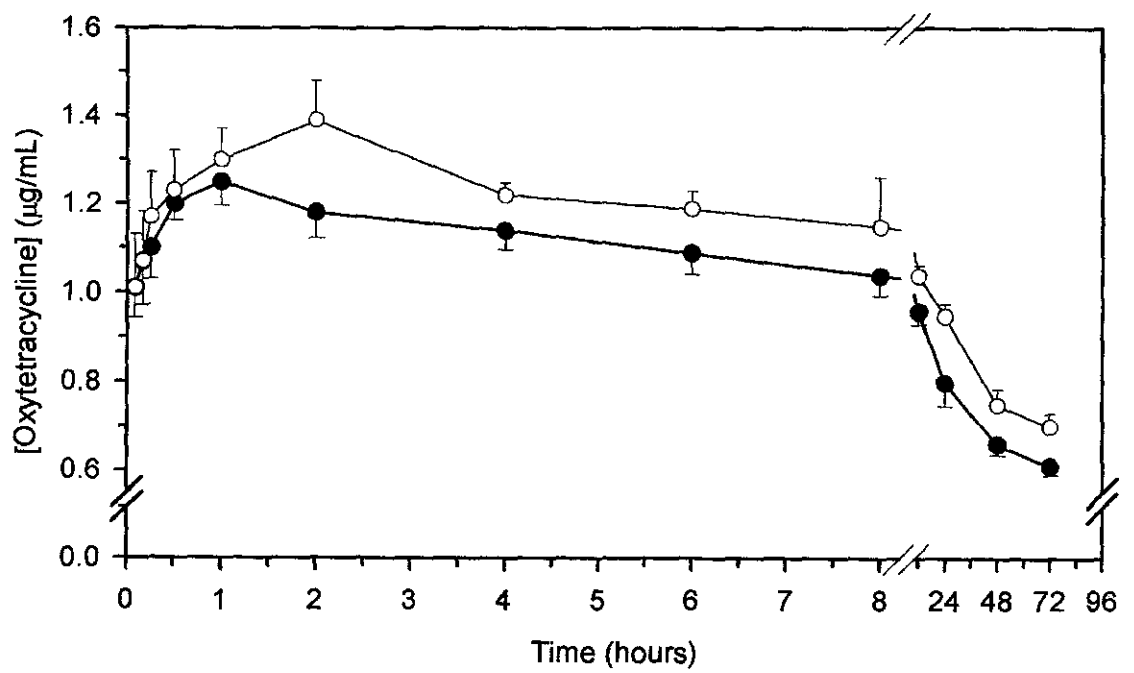


Figure 2: Mean serum concentrations of oxytetracycline (μg/ml) in 5 lactating goats that received an intramuscular injection of a long acting oxytetracycline formulation (30 mg/kg BW) with (open circles) or without (filled circles) an intramuscular injection of diminazene aceturate (3.5 mg/kg BW) 2 h previously. Values are mean ± SD.



DISCUSSION

The main finding of the study reported here in lactating goats was that concomitant administration of a new LA-OTC formulation and diminazine aceturate resulted in higher serum OTC concentrations than that occurring after administration of LA-OTC alone. This result was most likely due to decreased binding of OTC to plasma proteins in the presence of diminazine, although decreased binding to OTC vehicle (dimethylacetamide) or tissue may have also been present. Because the increase in serum OTC concentration following combined treatment was within the therapeutic range, the pharmacokinetic interaction between diminazine and LA-OTC is clinically relevant.

Intramuscular administration of a LA-OTC formulation (30 mg/kg BW) resulted in a maximal serum OTC concentration of 1.25 µg/ml at 1 hour (Figure 2) and a serum OTC concentration greater than 0.5 µg/ml (the MIC for most pathogenic bacteria) for at least 72 hours. The maximal serum OTC concentration achieved by the LA-OTC formulation was lower than that obtained previously in goats (3.27 µg/ml, Arqum et al., 1995, 3.44 µg/ml, Escudero et al., 1996) following intramuscular administration of a different LA-OTC formulation at 20 mg/kg BW.

The mean value for Vd_{area} (22.0 L/kg) in this study was markedly higher than those obtained in studies following intravenous injection of a different LA-OTC formulation (20 mg/kg BW) in goats (Vd_{area} = 0.95 L/kg; Mandall et al., 1992). The unusually high value of Vd_{area} in our study suggests that OTC was extensively bound in vivo or distributed into the peripheral compartment and explains why lower serum OTC concentrations were achieved than that observed by injection of a different LA-OTC formulation at 20 mg/kg BW in goats (Mandall et.al., 1992). Long acting antimicrobial formulations contain vehicles or adjuvants, such as polyvinylpyrrolidone, N-methylpyrrolidone, dimethylacetamide, and glycerol-formaldehyde that result in slow release of unbound drug and sustained antimicrobial concentrations above the target minimum inhibitory concentration (Neuschl et al., 2007). It is therefore likely that dimethylacetamide bound a significant portion of OTC in vivo after IV or IM injection, and that this contributed to the high Vd_{area}. Alternatively, the high Vd_{area} and low serum OTC concentrations may have been due to use of a bacterial growth assay to measure serum OTC concentrations, as this assay measures unbound OTC and not total OTC. Bacterial growth inhibition has been widely used to measure antimicrobial concentrations in serum or plasma because the assay is inexpensive and has clinical relevance because it measures

unbound (active) antimicrobial concentration as well as measuring antimicrobial metabolites that may also inhibit bacterial growth. However, bacterial growth may be inhibited or promoted by solvents or other agents in commercial formulations; the presence of growth promotants will result in measured values for serum concentration that are less than the true value. It is therefore possible that products in the LA-OTC formulation may have promoted bacterial growth in the assay used to measure time dependent changes in serum OTC concentration. However, growth promotion by the LA-OTC formulation appears to be an unlikely cause for the high Vd_{area} because we obtained similar OTC concentrations when measured by microbiologic and spectrophotometric assays.

The mean clearance of OTC in the new LA-OTC formulation administered to lactating goats in this study was 0.65 (ml/kg)/h. Lower mean clearance values for OTC of 0.11 (ml/kg)/h were previously recorded in goats (Rule et al., 2001) and sheep (Guimera, 1992) following IV injection of a different LA-OTC formulation. The mean elimination half-time of the LA-OTC formulation administered by single intramuscular injection to the lactating goats in the study reported here (24.8 hours) was within the range of other estimates in goats (39.0 hours, Arqum et al., 1995; 14.4 hours Payne et al., 2002).

The mean absorption half-life $t_{0.5(ab)}$ (17 mins) obtained in the present study following IM administration of the new LA-OTC formulation was faster than values of 1.43 hr reported in goats (Escudero et al., 1996) following IM administration of a different LA-OTC formulation. The long MRT (35.7 ± 4.5 h) in the present study also suggest that OTC was continuously released in to the circulation from its deposition site over a much longer time interval than that indicated by the $t_{0.5(ab)}$ value recorded in a previous study in calves; in that study OTC was absorbed in two phases following IM administration, 14% of the available dose was quickly absorbed with a biological half-life of 48 mins and 38% was absorbed more slowly with a half-life of 18 h (Toutain and Raynaud, 1983). These results suggest that some of the drug was available for immediate absorption as indicated with low C_{max} value determined in the present study but that the remainder acted as a depot and was absorbed slowly over next 72 h. This result is in agreement with the results obtained by Escudero et al., (1994) in goats after IM administration of conventional and long-acting formulations.

The results of the study reported here indicate that, based on a target MIC of 0.5

μg/ml, an effective serum OTC concentration is obtained for 48 to 72 h after IM injection of the new LA-OTC formulation at 30 mg/kg BW. Effective milk concentrations were present for 24 to 48 h, and effective urine concentrations were detected for 96 to 120 h after IM injection. On this basis, the new LA-OTC formulation could be administered at 30 mg/kg BW every 2-3 days when treating systemic bacterial infections in goats. A single IM injection of LA-OTC could be effective in the treatment of bacterial urinary infections in goats. Milk of lactating goats treated with the new LA-OTC formulation should be discarded for at least 5 days after a single IM dose in order to minimize the risk of OTC residues for human consumers. Co-administration of the new LA-OTC formulation and diminazene aceturate results in increased concentration of unbound OTC in serum.

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REFERENCES

Aliu, Y.O. and Odegaard, S. (1985): "Pharmacokinetics of diminazene in sheep". J.Pharmacok.Biopharm., 13 (2):173-184.

Aliu YO, Odegaard S, Signen E. (1984) Dimiazene/berenil: bioavailability and disposition in dairy goats. Acta vet. Scand; 25:593-596.

Anika SM, Nouws JFM, Van Gogh H et al. (1986): Chemotherapy and pharmacokinetics of some antimicrobial agents in healthy dwarf goats and those infected with *Ehrichia phagocytophila* (tick-borne fever). Rev. Vet. Sci.; 41:386-390.

Arqum, I.A.; Nawaz, M.; Khan, F.H. and Khalid, R. (1995): "Bioavailability and Disposition kinetics of two long-acting oxytetracycline after a single intramuscular administration in goats". Pakistan Vet. J, 15 (2): 51-53.

Arret, B.; Johnson, D.P. and Kirshboum, A. (1971): "Outline of details for microbiological assay of antibiotics, a second revision". Pharmacol.Sci., 60: 1689-1694.

Aronson, A.L. (1980): Pharmacotherapeutics of the vewer tetracyclines AVMA,167

Baggot, J.D. (1978): "Some aspects of clinical pharmacokinetics in veterinary medicine I. J. vet. Pharmacol. Therap., 1: 5-18.

Baxter, P. & McKellar, Q.A. (1990) Distribution of oxytetracycline in normal and diseased ovine lung tissue. J. vet. Pharm. Ther., 13, 428–431.

Cornwell, R.L. (1980) Evaluation of a long-acting injectable oxytetracycline. Mod. Vet. Pract., 61, 954-957.

Craigmill, A.L.; Holland, R.E.; Robinson.; Wetzlich, S. and Arndt, T. (2000): "Serum pharmacokinetics of oxytetracycline in sheep and calves and tissue residues in sheep following a single intramuscular injection of long-acting preparation" J. Vet. Pharmacol. Therap., 23 (6):345-352.

EL-Banna, H.A.; EL-Sooud, K.A. and Soliman, G.A. (1998): "Comparative pharma-

cokinetics of diminazene in lactating goats and sheep". J.Vet.Med.A., 46:49-57.

Escudero, E.; Carceles, C.M. and Serrano, J.M. (1994): "Pharmacokinetics of oxytetracycline in goats: modification induced by a long-acting formulation".Vet. Rec., 135: 548-552.

Escudero, E.; Carceles, C.M.; Ponferrada, C. and Baggot, J.D. (1996): Pharmacokinetics of a long-acting oxytetracycline formulation in sheep and goats". J. vet. Pharmacol. Therap., 19:75-77.

Fofana, A., Toma, L., Moran, D., Gunn, G.J. and Stott, A.W. (2009): Measuring the economic benefits and costs of Bluetongue virus outbreak and control strategies in Scotland. The 83rd Annual Conference of the Agricultural Economics Society, Dublin, 30th March to 1st April.

Fourtillan, J.B., Ingrand, I. & Moreau, J. (1989): Biodisponibilites de l'oxytetracycline chez les bovins apres administrations intramusculaire de deux formes a liberation controlee. Recueil de Med Veter, 165, 727±731.

Guimera, M.E. (1992): "Modificaciones del comportamiento farmacocinetico de la oxitetraciclina por sales metalicas. Incidencia clinica de la administracion de oxitetraciclina-calcio por vias intravenosa e intramuscular en ovinos". PhD Thesis. Facultad de Veterenaria. University of Cordoba, Spain.

Horspool, L.J.I. and Mc-Kellar, Q.A. (1990): "Disposition of oxytetracycline in horses, ponies and donkey after intravenous administration". Eq. Vet. J., 22: 284-285.

Jennings, F.W.; Murray, P.K. ;Murray, M.; Urquhart, G.M. (1974): Anaemia in trypanosomiasis: studies in rats and mice infected with Trypanosoma brucei. *Res Vet Sci.* Jan;16(1):70–76.

Khan M.A.; Muzammil S.; Musarrat J. (2002): Differential binding of tetracyclines with serum albumin and induced structural alterations in drug-bound protein. In. J. Biol. Macromol. 30 (5):243-249.

Klatt, P. and Hajdu, P. (1976): "Pharmacokinetic investigations on diminazene and rolitetracycline in comparison to a combination of both". Vet. Rec., 99 (19):372-374.

Lorian, V. (1980): "Antibiotics in laboratory medicine". U.S.A., 205-297.

Mamman, M.; Aliu, Y.O. and Peregrine, A.S. (1993): "Comparative pharmacokinetics of diminazene in non infected Boran cattle and Boran cattle infected with Trypanosoma congolense". Antimicrob. Agents Chemother.37 (5):1050-1055.

Mandal, T.K.; Sarkar, S.; Chakraborty, A.K.and Ghose, R.K. (1992): "Pharmacokinetics of oxytetracycline in presence of calcium gluconate in goats". Indian. J. Physiol. Pharmacol., 36 (2):112-114.

McElroy, D.E.; Ravis, W.R. and Clark, C.H. (1987): "Pharmacokinetics of oxytetracycline hydrochloride in rabbits". Am.J.Vet.Res., 48: 1261-1263.

Meijer, L.A.; Ceyssens, K.G.F.; de Jong, W.Th. and de Greve, B.I.J.A.C. (1993): "Three phase elimination of oxytetracycline in veal calves; the presence of an extended terminal phase". J. vet. Pharmacol. Therap., 16: 214-222.

Mevius, D.J.; Nouws, J.F.M.; Breukink, H.J.; Vree, T.B.; Driessens, F. and Verkaik, R. (1986): "Comparative pharmacokinetics, bioavailability and renal clearance of five parenteral oxytetracycline-20% formulations in dairy cows". Vet. Quart., 8: 285-294.

Molyneux, D.H.; Pentreath, V.; Doua, F. (1996): **African trypanosomiasis in man.** In *Manson's Tropical Diseases.* 20th edition.

Edited by: Cook GC. London: W.B. Saunders Company Ltd;1171-1196.

Moreno, L.; Serrano, J.M.; Reja, A.; Guimera, E. and Escudero, E. (1996): "Pharmacokinetics of oxytetracycline after intravenous administration at two dose levels to hens". J. vet. Pharmacol. Therap., 19: 495-497.

Neuschl J, Popelka P, Nagy J et al. Comparison of the serum level and pharmacokinetic parameters of oxytetracycline after administration of long action preparations in sheep. Medycyna Wet. 2007; 63(3):294-297.

Nouws, J.F.M.; Breukink, H.J.; Binkhorts, G.J.; Lohuis, J.; Van Lith, P.; Mevius, D.J.; and Vree, T.B. (1985): "Comparative pharmacokinetics and bioavailability of eight parenteral oxytetracycline-10% formulations in dairy cows". Vet. Quart., 7: 306-314.

Payne, M.A.; Babish, J.G.; Bulgin, M.; Lane, M.; Wetzlic,S. and Craigmill, A.L. (2002): "Serum pharmacokinetics and tissue and milk residues of oxytetracycline in goats following a single intramuscular injection of long-acting preparation and milk residues following a single subcutaneous injection". J. vet. Pharmacol. Therap., 25 (1):25-32.

Ponferrada, C.J. (1987): "Farmacocinetica de la oxitetraciclina en ovinos. Modificaciones inducidas por los preparados `Long-Acting`".Ph.D Thesis. University of Cordoba, Spain.

Rolan, P. E. (1994): Plasma protein binding displacement interactions—why are they still regarded as clinically important? Br J Clin Pharmacol.; 37:125–128.

Rule, R.; Moreno, L.; Serrano, J.M.; Roman, A.G.; Moyano, R. and Garcia,J. (2001): Pharmacokinetics and residues in milk of oxytetracycline administered parenterally to dairy goats". Aust. Vet. J., 79 (7):492-496.

Riviere, J.E. (1999): Comparative Pharmacokinetics Principles Techniques And Applications, Iowa State Press.

Silayo, R.S., Mamman, M., Moloo, S.K., Aliu, Y.O., Gray, M.A. & Peregrine, A.S. 1992. *Res.* Vet. Sci., 53: 98-105

Riviere, J.E. & Spoo, J.W. (1995) Tetracycline antibiotics. In Veterinary Pharmacology and Therapeutic. 7th ed. Ed. Adams, R.H. Iowa State University Press, Ames, Iowa, pp. 785-796.

Snell F.D, & Snell C.T. (1971) Colorimetric methods of analysis, New York: V.N. Reinhold Company, Vol. IVAAA,:216.

Toutain, P.L. and Raynaud, J.P. (1983): "Pharmacokinetics of oxytetracycline in young cattle: comparison of conventional vs. long-acting formulation". Am. J. Vet. Res., 44(7):1203-1209.

Varma, K.J.and Paul, B.S. (1983): "Pharmacokinetics and plasma protein binding of oxytetracycline in buffalo". Am. J. Vet. Res., 44(3):497-499.

Walton, J. R. 1983. Antibiotics, animals, meat and milk. Zbl. Vet. Med. A. 30, 81-92.

Yamaoka, K.; Nakagawa, T. and Uno, T. (1978): "Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations". J. Pharmacok. Biopharm., 6: 165-175.

Xia, W.; Neilsen, P. and Gyrd-Hensen, N. (1983): "Comparison of pharmacokinetic parameters for two oxytetracycline preparations in cows". Acta Vet. Scand., 24: 120-128.

تأثير الداى مينازين اسيتيورات على المسار الحركى لتركيبة الأوكسى تتراسيكلين طويل المفعول فى الماعز الحلوب

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يعتبر الأوكسى تتراسيكلين و الداى مينازين اسيتيورات من أكثر الأدوية المستخدمة فى علاج حالات العدوى المزدوجة من البكتيريا و طفيليات الدم الأولية فى المجترات.

أجريت هذه التجربة لدراسة المسار الحركى لتركيبة جديدة من الأوكسى تتراسيكلين طويل المفعول بعد الحقن عن طريق العضل و الوريد و كذلك مدى تأثير التعاطى المتزامن مع الداى مينازين اسيتيورات على المسار الحركى.

اجريت الدراسة على عدد عشرة ماعز حلوب من النوع البلدى، حيث تم اعطاء الأوكسى تتراسيكلين على جرعات يفصل بينها اسبوعين على التوالى لضمان خلو أجسام الحيونات من الدواء.

أعطى الأوكسى تتراسيكلين بجرعة واحدة (٣٠مجم/ من وزن الحيوانات) عن طريق الحقن العضلى أو الوريدى فى الحيوانات الغير معالجة بالداى مينازين اسيتيورات (٣.٥مجم / كجم من وزن الحيوانات ساعتين قبل إعطاء الأوكسى تتراسيكلين).

تم اخذ عينات بول و دم و لبن على فترات مختلفة لقياس تركيز الأوكسى تتراسيكلين بها باستخدام الطريقة الميكروبيولوجية و قياس مدى ارتباط الدواء مع بروتينات الدم و البول و اللبن باستخدام الترشيح الدقيق.

اوضحت النتائج ان اعطاء الداى مينازين يؤدى الى زيادة تركيز الأوكسى تتراسيكلين فى مصل الدم و كان اعلى تركيز (1.25 ± 0.02 $\mu g.ml^{-1}$ and 1.39 ± 0.04 $\mu g.ml^{-1}$) عند (1.8 ± 0.3 hours and 2.4 ± 0.4 hours) ، و استمر الدواء بتركيز مؤثر فى اللبن حتى ٢٤ و ٤٨ ساعة و فى البول حتى ٩٦ و ١٢٠ ساعة الحيوانات الغير معالجة و المعالجة بالداى مينازين على التوالى. و اوضحت النتائج ان للدواء قدرة متوسطة على الإتحاد مع بروتينات اللبن، البول و الدم.

و يستخلص من هذه الدراسة ان الإعطاء المتزامن للأوكسى تتراسيكلين و الداى مينازين يعطى نتائج يمكن ان تساعد فى زيادة كفاءة الدواء العلاجية.