Iranian Journal of Veterinary Research, Shiraz University, Vol. 9, No. 2, Ser. No. 23, 2008

The pharmacokinetics and milk residual behaviour of tylosin in lactating Najdi ewes

Al-Wabel, N. A.

Department of Veterinary Medicine, Faculty of Agriculture and Veterinary Medicine, Al-Qassim University, Buraidah 51431, P.O. Box 1482, Saudi Arabia

Correspondence: N. Al-Wabel, Department of Veterinary Medicine, Faculty of Agriculture and Veterinary Medicine, Al-Qassim University, Buraidah 51431, P. O. Box 1482, Saudi Arabia. E-mail: naseralwabel@ yahoo.com

(Received 30 Apr 2007; revised version 4 Sept 2007; accepted 9 Oct 2007)

Summary

The objective of this study was to evaluate kinetics and the residual decline of tylosin in milk and plasma of lactating Najdi ewes following single intramuscular injection of tylosin at the dose of 10 mg/kg. Blood and milk samples were collected from the ewes before and at different time intervals after treatment. Tylosin concentrations were determined by microbiological agar plate assay using *Bacillus subtilis* ATCC 6633 as the test organism. The pharmacokinetic parameters were processed using the methods of least square and statistical moments. The plasma levels of tylosin against time were adequately described by a one-compartment open model. The mean obtained values indicated a fairly low area under the plasma concentration-time curve (AUC) (3.0 μg.h/ml) and the maximum plasma concentration (C_{max}) of 0.63 μg/ml with T_{max} of 1.33 h. The plasma elimination half-life ($t_{1/2el}$) and the mean residence time (MRT) were 2.3 h and 3.9 h, respectively. A different pattern was shown for milk, in which measurable residual levels are found in all animals up to 72 h after treatment. The mean value of milk AUC was 88.1 μg.h/ml and the $t_{1/2el}$ was 3.3 h. *In vitro* mean plasma and milk proteins binding of tylosin were 19.3 and 30.2%, respectively. The milk withdrawal period of tylosin in lactating Najdi ewes should be at least 72 h to avoid risks in consumers.

Key words: Tylosin, Pharmacokinetics, Milk residues

Introduction

Milk residues of antimicrobials represent a potential risk to the consumer and may lead to allergic reactions, changes in the pattern of intestinal flora, and finally bacterial resistance, rendering antibiotic treatment ineffective (Dewdney *et al*., 1991; Currie *et al*., 1998). Important losses are also aggravated the fermented products by inhibiting the bacterial processes involved in the elaboration of cheese and cultured milk products (Brady and Katz, 1988). Tylosin is a macrolide antibiotic that inhibits bacterial protein synthesis by blocking the translocation step (Brisson-Noel *et al*., 1988). It is active against Gram-positive bacteria, anaerobic bacteria and mycoplasma. Moreover, it treats pneumonia, foot rot, metritis and dysentery in farm animals (Gutiérrez and Rodríguez, 1993). The pharmacokinetics of tylosin has been described in a variety of animals including calves (Burrows *et al*., 1983), dogs (Duthu, 1985), goats (Atef *et al*., 1991) and avian species (Locke *et al*., 1982). Little information is available on milk residual pattern of tylosin especially in Najdi ewes. The aims of the present study were to determine tylosin levels in plasma and milk and several pharmacokinetic parameters after single intramuscular injection of the drug in lactating Najdi ewes and to evaluate the residual of tylosin in milk.

Materials and Methods

Animals

The trial was conducted on five lactating Najdi ewes, with body weight of 40-50 kg. The ewes were kept indoors in an experimental animal shed, according to the standard operating husbandry with the permission of Animal Care Committee in

Al-Qassim University. They were fed with concentrated diet and hay and received water *ad libitum*. All animals were clinically healthy and had not been treated with antibiotics in the last 30 days before the trial. Animals were given a single dose of 10 mg/kg BW of tylosin (Tylan®, Elanco, Eli lilly, Geneva, Switzerland, solution containing tylosin base at a concentration of 200 mg/ml) intramuscularly into the left gluteal muscles. Blood samples were collected from the right jugular vein into heparinized tubes just before and at 5, 10, 20, 30, 45 min and 1, 2, 4, 6, 8, 10, 12 and 24 h after intramuscular injections. The blood was centrifuged immediately at 1500 g for 15 min to obtain clear plasma which was kept at -20°C until analysis. Milk samples were collected by hand milking in sterilized containers before and at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 18, 24, 48, 72 and 96 h after injections. Complete evacuation of the udder was done after each sample. The samples were frozen promptly and stored at -20°C until analysis. Tylosin concentrations in plasma and milk were determined by the microbiological agar diffusion assay (Tsai and Kondo, 2001) using *Bacillus subtilis* ATCC 6633 as the test organism. This method of analysis does not differentiate between tylosin and its metabolites. Standard curves were prepared in pooled antibacterial-free plasma and milk. 0.1 ml of all samples were placed directly into the wells of the Mueller-Hinton agar plate without any clean-up step. The lower limit of quantitation by this method was 0.07 µg/ml in plasma and milk. The calibration curves were linear over the range of concentrations between 0.07-25 µg/ml with a correlation coefficient (r^2) of 0.987 and the intra-assay coefficient of variation (CV) was 7%.

The extent of *in vitro* protein binding was determined using the method of Craig and Suh (1980) which is based on the diffusion of the free antibiotic into the agar medium. To estimate the protein binding of tylosin, the drug was dissolved in phosphate buffer and in antibiotic-free plasma and milk of ewes at concentrations of 0.312, 0.625, 1.25, 2.5 and 5 µg/ml. The differences in the diameter of the inhibition zone between the solutions of the drug in the buffer and plasma and milk samples were calculated as a per cent of protein bound fraction according to the following equation:

$$\text{Protein binding \%} = \frac{\text{zone of inhibition in b - zone of inhibition in p} \times 100}{\text{zone of inhibition in b}}$$

b = buffer and p = plasma

Pharmacokinetic analysis

A computerized curve-stripping programme (Rstrip, Micromath Scientific Software, version 5.0, Salt Lake City, UT, USA) was used to analyse the concentration-time curves for each individual lactating ewes after intramuscular administration. The curve that expresses the decline in tylosin concentrations, as a function of time was best fitted by a one-compartment open model. Statistical moments (Yamaoka *et al*., 1978) were also used to compute the non-compartmental models parameters of peak concentration (C_{max}), time to the peak concentration (T_{max}) and mean residence time (MRT). In addition, C_{max} and T_{max} were observed directly from the plotted curve. The area under the plasma concentration-time curve (AUC) and area under the first moment curve (AUMC) were calculated by the trapezoidal method by extrapolation to infinity. Mean residence time was calculated as MRT = AUMC/AUC. The extent of the drug penetration from blood into milk was expressed by the AUC_{milk}/AUC_{plasma} and $C_{max.milk}/C_{max.plasma}$ ratios (Ziv *et al*., 1995). The estimated pharmacokinetic parameters for tylosin in plasma and milk are reported as mean ± SEM. The Student's t-test was used to compare the concentrations and kinetic parameters in plasma and milk. The values were considered statistically significant at $p \leq 0.05$.

Results

The concentrations of tylosin, injected as a single dose, in plasma and milk of ewes are illustrated in Fig. 1. The present investigation revealed that the plasma concentration-time curve of tylosin was best fitted to one-compartment open model. Tylosin was detected in plasma 15 min post-injection and no amount of tylosin could be

detected in plasma 10 h following intramuscular injection. Concentration of tylosin in milk was higher than in plasma and it was detected within 0.5 h post-injection (0.4 µg/ml) then increased gradually to reach a peak ($C_{max.obs}$) of 11.0 µg/ml, 6 h after treatment (Fig. 1). Measurable residual levels are found in milk of all animals up to 72 h after treatment. The mean calculated values indicated plausible low plasma AUC of 3.0 µg.h/ml and C_{max} of 0.63 µg/ml with T_{max} of 1.3 h. The plasma elimination half-life ($t_{1/2el}$) was 2.3 h and the MRT was 3.9 h. Tylosin was slowly eliminated from milk as indicated by its long $t_{1/2el}$ and MRT, 3.3 h and 7.83 h, respectively. The ratios of $C_{max.milk}/C_{max.plasma}$ and AUC_{milk}/AUC_{plasma} were 11.8 and 29.5, respectively. These high values indicated privileged penetration of tylosin from the bloodstream to the mammary gland of lactating ewes following intramuscular administration. Selected pharmacokinetic parameters of tylosin in plasma and milk are depicted in Table 1.

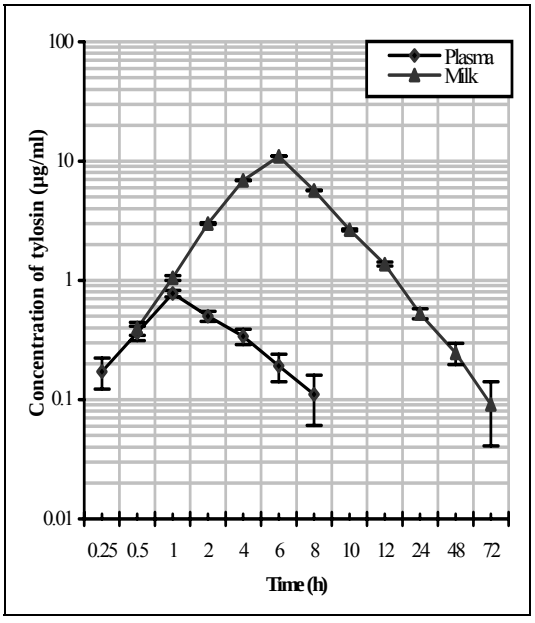

Fig. 1: Plasma and milk concentrations (Mean ± SEM, n = 5) vs. time curves for tylosin after intramuscular injection in Najdi ewes at a dose of 10 mg/kg

The mean plasma and milk protein binding of tylosin *in vitro* were 19.3 and 30.2%, respectively. It was clear that the protein binding affinity is inversely correlated to the drug concentrations (Table 2).

Table 1: Mean of selected pharmacokinetic parameters of tylosin in Najdi ewes following a single intramuscular injection of 10 mg/kg BW (n = 5)

Kinetic parameters	Unit	Plasma	Milk
Observed C_{max}	µg/ml	0.74 ±0.13	11.0 ±1.3***
Calculated C_{max}	µg/ml	0.63 ±0.11	7.41 ±1.39**
Observed T_{max}	h	1.0 ±0.2	6.0 ±1.1**
Calculated T_{max}	h	1.3 ±0.8	4.5 ±0.9*
Lag time	h	0.16 ±0.06	---
K_{ab}	/h	1.85 ±0.33	---
$t_{1/2ab}$	h	0.38 ±0.10	---
K_{el}	/h	0.301 ±0.07	0.21 ±0.07
MRT	h	3.9 ±0.87	7.83 ±1.13*
$t_{1/2el}$	h	2.3 ±0.7	3.3 ±0.7
AUC	µg.h/ml	3.0 ±0.8	88.1 ±8.6***
AUMC	µg.h^2/ml	11.4 ±1.8	613.5 ±54.4***
Calculated $C_{max-milk}/C_{max-plasma}$			11.8
AUC_{milk}/AUC_{plasma}			29.5

C_{max}: peak drug concentration, T_{max}: time to the peak concentration, K_{ab}: absorption rate constant, $t_{1/2ab}$: absorption half-life, $t_{1/2el}$: elimination half-life, MRT: mean residence time, K_{el}: first-order elimination rate constant, AUC: area under the curve from zero to infinity by the trapezoidal integral, and AUMC: area under the first moment curve from zero to infinity by the trapezoidal integral. *, **, *** = Significant at P<0.05, P<0.01 and P<0.001, respectively compared with plasma. --- Not applicable

Table 2: *In vitro* protein binding of tylosin in Najdi ewes following a single intramuscular injection of 10 mg/kg BW (n = 5)

Concentrations (µg/ml)	% of protein binding	
	Plasma	Milk
5	12.0 ± 1.3	21.0 ± 2.1**
2.50	16.9 ± 0.8	26.3 ± 1.9**
1.25	20.5 ± 1.3	30.5 ± 2.1**
0.63	22.0 ± 1.3	34.5 ± 1.5***
0.31	25.1 ± 1.1	38.8 ± 2.1***
	19.3 ± 2.3	30.2 ± 3.1*

*, **, *** = Significant at P<0.05, P<0.01 and P<0.001, respectively compared with plasma

Discussion

The use of antimicrobial agents in animals increases the possibility of transfer of resistant genes from animal's bacterial isolates to human's bacterial isolates (Murray, 1998). The widespread use of tylosin in animals hastens the resistance among pathogenic strains to macrolides in human and augments the cross-resistance with erythromycin (Roberts *et al*., 1999). According to Lakritz *et al*. (1999), the assessment of macrolide antibiotics using microbiological assay is equivalent to the high-performance liquid chromatography without a significant difference. Microbiological assay has also been used recently to determine the concentrations of tylosin in milk (Litterio *et al*., 2007). This bioassay does not differentiate between tylosin and its active metabolites. It has been reported that in rabbits, tylosin has a microbiologically active metabolite, N-demethylation of tylosin (Carletti *et al*., 2003). This metabolite has not been reported in other species. When determining a dosage regimen of a drug, it is adequate to measure total antimicrobial activity without differentiation between the activity of the parent drug and the metabolite (Dowling *et al*., 1995). The experimental data of observed plasma concentrations revealed that pharma-cokinetics of tylosin was adequately described by one-compartment open model since the distribution phase was hidden by the absorption phase. This model has also been used by various workers to describe the pharmacokinetics of macrolides in goats (Atef *et al*., 1991) and cattle and buffaloes (Saurit *et al*., 2002). Tylosin was rapidly absorbed following intramuscular administration as reflected by an absorption half-life of about 0.4 h. Data in the present study showed that the calculated C_{max} in plasma was 0.63 µg/ml which is close to that observed in cattle (0.64 µg/ml) by Saurit *et al*. (2002). However, this value was lower than those reported in pigs (1 µg/ml, Prats *et al*., 2002), chickens (1.2 µg/ml, Kowalski *et al*., 2002) and goats (2.38 µg/ml, Atef *et al*., 1991). The plasma $t_{1/2el}$ was 2.3 h, that is much lower than those reported in pigs (24 h, Prats *et al*., 2002) and for tilmicosin in lactating ewes (15.4 h, Atef *et al*., 1999). This variation may be attributed to the anatomical and physiological differences among various animal species. Tylosin concentrations in milk were much higher than those of plasma, and the observed C_{max} was reached 11.0 µg/ml, 6 h after treatment. These results are in agreement with those reported for tilmicosin in sheep (Parker *et al*., 1994). They found that the mean C_{max} of tilmicosin in serum and milk were 1.36 and 10.25 µg/ml, respectively. Parenterally administered antibiotics are distributed throughout the body by blood and diffuse to target tissues to exert systemic effects and reaching other non-targeted tissues such as the mammary glands. Drug concentrations attained in different tissues depend on the ability of the drug to penetrate the capillary endothelium and to diffuse across biological membranes of lipid nature (Baggot, 1977). The degree of drug ionization in milk and plasma, its lipid solubility, and the extent to which the drug binds to the milk and plasma proteins determines its concentrations in the milk and in the bloodstream (Ziv and Sulman, 1973). In general, only unbound, non-ionized lipid-soluble molecules reach the mammary glands and are excreted in milk. Tylosin is a high lipid-soluble organic base (pKa = 7.1) with 30% binding to milk proteins, and has a low degree of ionization (Gingerich *et al*., 1977). It is, thus, widely distributed throughout udder tissues and milk. A similar mechanism was anticipated for spiramycin, as a macrolide antibiotic in milk (Nouws and Ziv, 1980). The ratios of $C_{max.milk}/C_{max.plasma}$ and AUC_{milk}/AUC_{plasma} were indicative of a privileged penetration of tylosin from the bloodstream to the mammary gland of lactating ewes following intramuscular administration and support its use in mastitis with systemic reactions.

The capacity of tylosin to be bound to plasma and milk proteins of ewes was 19.3 and 30.2%, respectively, which is consistent with that reported for tilmicosin in Barki ewes (Atef *et al*., 1999). They found that binding of tilmicosin to plasma and milk proteins was 16.8 and 26.8%, respectively. Milk proteins binding to the drug is higher than that of plasma as the casein fraction is a major binding component in milk for all

tested drugs (Stebler and Guentert, 1990). This wide range of values may be due to the different methods used for determination of protein binding tendency. No tylosin concentrations could be detected in any of milk samples 72 h post-injection. Accordingly, the milk withdrawal period of tylosin in Najdi lactating ewes should be at least 72 h. Further studies are desirable to estimate the tissue concentrations of tylosin in Najdi sheep using different dosage regimens.

References

Atef, M; Abo El-Sooud, K; Nahed, E and Tawfik, M (1999). Elimination of tilmicosin in lactating ewes. Dtsch. Tierarztl. Wochenschr., 106: 291-294.

Atef, M; Youssef, SA; Atta, AH and EL-maaz, AA (1991). Disposition of tylosin in goats. Dtsch. Tierarztl. Wochenschr., 98: 451-453.

Baggot, JD (1977). *Principles of drug disposition: the basis of veterinary clinical pharmacology*. 1st Edn., Philadelphia, W. B. Saunders Co., PA. PP: 1-134.

Brady, MS and Katz, SE (1988). Antibiotic/antimicrobial residues in milk. J. Food Prot., 51: 8-11.

Brisson-Noel, A; Trieu-Cuot, P and Courvalin, P (1988). Mechanism of action of spiramycin and other macrolides. J. Antimicrob. Chemother., 22: 13-23.

Burrows, GE; Barto, PB; Martin, B and Tripp, ML (1983). Comparative pharmacokinetics of antibiotics in newborn calves: chloramphenicol, lincomycin and tylosin. Am. J. Vet. Res., 44: 1053-1057.

Carletti, M; Gusson, F; Zaghini, A; Dacasto, M; Marvasi, L and Nebbia, C (2003). *In vitro* formation of metabolic-intermediate cytochrome P450 complexes in rabbit liver microsomes by tiamulin and various macrolides. Vet. Res., 34: 405-411.

Craig, AW and Suh, B (1980). Protein binding and the antibacterial effects. Method for determination of protein binding. In: Lorian, V (Ed.), *Antibiotics in laboratory medicine*. (1st Edn.), Williams and Wilkins, Baltimore, MD. PP: 265-297.

Currie, D; Lynas, L; Kennedy, G and McCaughey, J (1998). Evaluation of modified EC four-plate method to detect antimicrobial drugs. Food Addit. Contam., 15: 651-660.

Dewdney, JM; Maes, L; Raynaud, JP; Blanc, F; Scheid, JP; Jackson, T; Lens, S and Verschueren, C (1991). Risk assessment of antibiotic residues of beta-lactams and macrolides in food-products with regard to their immunoallergic potential. Food Chem. Toxicol., 29: 477-483.

Dowling, PM; Wilson, RC; Tyler, JW and Duran, SH (1995). Pharmacokinetics of ciprofloxacin in ponies. J. Vet. Pharmacol. Therap., 18: 7-12.

Duthu, GS (1985). Interspecies correlation of the pharmacokinetics of erythromycin, oleandomycin, and tylosin. J. Pharm. Sci., 74: 943-946.

Gingerich, DA; Baggot, JD and Kowalski, JJ (1977). Tylosin antimicrobial activity and pharmacokinetics in cows. Can. Vet. J., 18: 96-100.

Gutiérrez Martin, CB and Rodríguez Ferri, EF (1993). In vitro susceptibility of *Pasteurella multocida* subspecies multocida strains isolated from swine to 42 antimicrobial agents. Zentralbl. Bakteriol., 279: 387-393.

Kowalski, C; Rolinski, Z; Zan, R and Wawron, W (2002). Pharmacokinetics of tylosin in broiler chickens. Pol. J. Vet. Sci., 5: 127-130.

Lakritz, J; Wilson, WD and Mihalyi, JM (1999). Comparison of microbiologic and high-performance liquid chromatography assays to determine plasma concentrations, pharmacokinetics, and bioavailability of erythromycin base in plasma of foals after intravenous or intragastric administration. Am. J. Vet. Res., 60: 414-419.

Litterio, NJ; Calvinho, LF; Flores, MM; Tarabla, HD and Boggio, JC (2007). Microbiological screening test validation for detection of tylosin excretion in milk of cows with low and high somatic cell counts. J. Vet. Med. A. Physiol. Pathol. Clin. Med., 54: 30-35.

Locke, D; Bush, M and Carpenter, JW (1982). Pharmacokinetics and tissue concentrations of tylosin in selected avian species. Am. J. Vet. Res., 43: 1807-1810.

Murray, BE (1998). Diversity among multidrug-resistant enterococci. Emerg. Infect. Dis., 4: 37-44 (Review).

Nouws, JFM and Ziv, G (1980). Distribution and residues of macrolide antibiotic in normal dairy cows. Arch. Lebensmittelhyg., 30: 202-208.

Parker, RM; Patel, RKP; Mclaren, IM and Francis, PG (1994). Residues of tilmicosin in milk of sheep after subcutaneous administration. *Proceeding of the European Association for Veterinary Pharmacology and Toxicology*, 6th International Congress. Edinburgh, UK. PP: 226-227.

Prats, C; El-Korchi, G; Francesch, R; Arboix, M and Perez, B (2002). Disposition kinetics of

tylosin administered intravenously and intramuscularly to pigs. Res. Vet. Sci., 73: 141-144.

Roberts, MC; Sutcliffe, J; Courvalin, P; Jensen, LB; Rood, J and Seppala, H (1999). Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. Antimicrob. Agents Chemother., 43: 2823-2830.

Saurit, AR; Rubio, M; Baroni, E; San, AM; Sanchez, S and Boggio, JC (2002). Some comparative aspects of the pharmacokinetics of tylosin in buffaloes and cattle. Vet. Res. Commun., 26: 49-54.

Stebler, T and Guentert, TW (1990). Binding of drugs in milk: the role of casein in milk protein binding. Pharm. Res., 7: 633-637.

Tsai, C and Kondo, F (2001). Improved agar diffusion method for detecting residual antimicrobial agents. J. Food Prot., 64: 361-366.

Yamaoka, K; Nakagawa, T and Uno, T (1978). Statistical moments in pharmacokinetics. J. pharmacokinet. Biopharm., 6: 547-558.

Ziv, G and Sulman, FG (1973). Penetration of lincomycin and clindamycin into milk in ewes. Br. Vet. J., 129: 83-91.

Ziv, G; Kurtz, B; Risenberg, R and Glickman, A (1995). Serum and milk concentrations of apramycin in lactating cows, ewes and goats. J. Vet. Pharmacol. Ther., 18: 346-351.