DISPOSITION KINETICS AND CLINICAL RESPONSE OF LONG ACTING OXYTETRACYCLINE IN BUFFALOES SUFFERING FROM SUBCLINICAL MASTITIS

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ABSTRACT

Disposition kinetics and clinical response of long acting oxytetracycline (Terramycin/La) 20mg/kg body weight intramuscular dose was investigated in buffaloes suffering from sub-clinical mastitis. The blood and milk samples were collected at different time intervals and the kinetic parameters were calculated with a computer program. Absorption half-life for plasma was 1.92 ± 0.78 hours (mean ± SD) and milk 7.17 ± 3.46 hours. Cmax in plasma was 7.36 ± 1.40 and in milk was 4.86 ± 1.90 μg/ml. After distribution equilibrium phase I half-life value was 3.92 ± 3.06 hours for plasma and 7.57 ± 3.23 hours for milk. The elimination half-life (Phase II) was 38.25 ± 18.85 for plasma and 63.34 ± 64.13 for milk. Total body clearance was 100 ± 20 ml. h^{-1}. kg^{-1} for milk. Somatic cells count in the mastitic buffaloes at the start of experiment was 4.46 ± 0.73 millions/ml milk. Following intramuscular injection of Terramycin/La, the cells counts on day 1, 2, 3, 4, 5, 12 and 19 were 4.25 ± 0.14, 3.21 ± 0.17, 2.27 ±0.41, 2.23 ± 0.26, 1.95 ± 0.18, 1.70 ± 0.23 and 1.33 ± 0.36 millions/ml milk. The cells count declined rapidly until day 3 after injection and was related to the plasma and milk levels of the drug, which persisted above MIC level during the 7-8 days observation period. The therapeutic level or minimum inhibitory concentration of the drug in milk and plasma was maintained until 84 hours.

INTRODUCTION

Mastitis is one of the most important limiting factors in economy of dairy units. Clinical cases show frank symptoms and are easy to diagnose. A large number of animals with subclinical mastitis are not readily detectable by routine clinical examination but require indirect laboratory test. From a clinical view-point successful clinical or subclinical mastitis are not readily detectable by routine clinical examination but require indirect laboratory test. From a clinical view-point successful clinical or subclinical mastitis therapy depends on effective passage of antibiotic from blood into milk and retaining its concentration in the mastitic udder for a considerable period. To achieve therapeutic concentrations, antibiotic(s) has to be injected repeatedly during the course of therapy. The prolonged effect of long acting oxytetracycline preparation is claimed to be due to the use of an aqueous 2-Pyrolidone based formulation which leads to a controlled precipitation of oxytetracycline at the site of injection from where it is absorbed slowly (Simpson 1978 and Cornwell, 1980). Thus, long acting oxytetracycline is a broad-spectrum antimicrobial preparation, which keeps promising efficacy for the treatment of mastitis and other diseases even after a single injection. The buffalo is a very important dairy animal in several Asian and some African countries with higher incidence of subclinical mastitis. The present study deals with the disposition kinetics and therapeutic effect of long acting oxytetracycline in buffaloes suffering from sub-clinical mastitis.

MATERIALS AND METHODS

Disposition kinetics and clinical response of long acting oxytetracycline were investigated in seven Nili-Ravi buffaloes suffering from sub-clinical mastitis. The animals with one or more quarters affected with mastitis were selected on the basis of clinical and laboratory examination. White side and sensitivity tests were performed to confirm the sub-clinical stage. The study was conducted during the months of October-November at the Livestock Experimental Station, University of Agriculture, Faisalabad. All the animals were maintained under similar management conditions. The average body weight of buffaloes was 550 kg, ranging

from 500 to 600 kg. Each buffalo was given a single intramuscular dose of long acting oxytetracycline (Terramycin/LA, Pfizer Labs. Pakistan) at a dosage level of 20-mg/kg body weight. The long-acting injectable Terramycin/LA contained 200 mg oxytetracycline/ml.

Disposition Kinetics

Animals were restrained in a stall for simultaneous blood and milk collections. After restraining the animal, a sterilized plastic venous cannula was inserted into the jugular vein for the collection of blood samples. Blank blood samples (zero samples) was collected prior to drug administration. While the other blood samples were collected in sterilized heparinized test tubes at 0.25, 0.5, 1, 2, 4, 12 and 24 hours and then at 24 hours interval up to 96 hours post drug injection. Immediately after collection, blood samples were centrifuged at 2000 rpm for 10 minutes and plasma was separated and stored at 4°C in refrigerator until analysis. Blank milk sample was collected before the administration of drug. Milk samples were collected in sterilized plastic vials at 0.5, 1, 1.5, 2, 3, 4, 6 and 12 hours and then 24 hourly upto 96 hours after the drug administration.

Concentration of oxytetracycline in plasma and milk was determined by microbiological assay (Disc agar diffusion method) using *Bacillus subtilus* as test organism (Arret *et al.* (1971).

The concentration of Terramycin/LA in plasma and milk versus time data from each experiment were analyzed separately for determination of absorption, distribution and elimination kinetic parameters by a PC computer program (MW\PHARM. Version 3.02, University Center for Pharmacy, Department of Pharmacology & Therapeutics, University of Gorningen, The Netherlands).

RESULTS AND DISCUSSION

The disposition kinetics and clinical response of Terramycin/LA was investigated in seven Nili-Ravi buffaloes suffering from sub-clinical mastitis after a single intramuscular administration of 20mg/kg dose. The average values for the concentration of Terramycin LA in plasma and milk at various time intervals following injections have been shown in Fig 1. From the Fig it is seen that the concentration of during first six hours after injection were higher in plasma than those in milk following which, the concentration in milk remained higher. At 84 hours the concentration was 0.77 μg/ml in milk and 0.50 μg/ml in plasma. The results show that the therapeutic level or minimum inhibitory concentration of the drug in milk and plasma was maintained until 84 hours. The minimum inhibitory concentration for most susceptible pathogens has been stated to be 0.5 mg/l (Escudero *et al.*, 1994).

The average ± SD values for the absorption kinetics of Terramycin/LA are presented in Tale 1. the drugs pass from plasma into milk by non-ionic diffusion through the mammary epithelium (Rasmussen, 1973. From the results it is seen that the absorption of long acting oxytetracycline from intramuscular site of injection into plasma is relatively a slow process with an absorption half-life 1.92 ± 0.78 hours (mean ± SD).

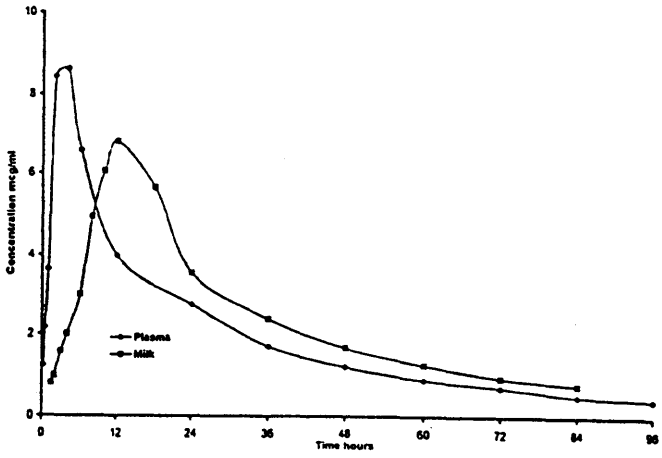

Fig. 1: Mean plasma and milk concentration of oxytetracycline LA in 7 mastitic buffaloes following 20 mg/kg b.wt intramuscular dose.

The passage of drug into milk was even slower with absorption half-life 7.17 ± 3.46 hours. The time of maximum absorption (Tmax) was nearly three times longer for milk than that for plasma. The maximum concentration of the drug Cmax for plasma was 7.36 ± 1.40 and for milk 4.86 ± 1.90 μg/ml. Since, oxytetracycline has a bacteriostatic action, the drug concentration during the course of therapy should not fall below the minimum inhibitory concentration (MIC) in the mammary tissue of mastitic udder. In cows, Nouws *et al.*, (1985) reported that a mean maximum milk OTC concentrations in the range between 0.92 and 1.43 μg/ml, were achieved 12 to 24 h p.i. The OTC milk concentration of the drug Cmax for plasma was

Table 1: Mean ± SD values for absorption kinetics of Terramycin/LA in plasma and milk of buffaloes suffering from subclinical mastitis following intramuscular administration of 20mg/kg b.wt dose.

Pharmacokinetics Parameters and units	Mean ± SD	
	Plasma	Milk
Kabs. h^{-1}	0.545 ± 0.574	0.129 ± 0.085
Absorption half-life hours	1.92 ± 0.78	7.17 ± 3.46
Lag time hours	0.14 ± 0.18	1.26 ± 0.49
Tmax hours	4.03 ± 1.61	12.44 ± 4.46
Cmax μg/ml	7.36 ± 1.40	4.86 ± 1.90
AUC Trapezoid h.mg/l	191.00±48.00	195.00 ± 96.00

Table 2: Mean ± SD values for disposition kinetics of Terramycin/LA in plasma and milk of buffaloes suffering from subclinical mastitis following intramuscular administration of 20mg/kg b.wt dose.

Pharmacokinetics Parameters and units	Mean ± SD	
	Plasma	Milk
A μg/ml	13.10 ± 4.25	8.74 ± 4.59
α h^{-1}	0.257 ± 0.131	0.119 ± 0.079
B μg/ml	3.46 ± 2.18	3.76 ± 4.11
ß h^{-1}	0.021 ± 0.008	0.025 ± 0.030
Half-life phase 1 hours	3.92 ± 3.06	7.57 ± 3.23
Half-life phase2 hours	38.25 ± 18.85	63.34 ± 64.13
MRT hours	40.67 ± 11.07	85.32 ± 80.43
Vc $l.kg^{-1}$	1.39 ± 0.65	1.85 ± 0.85
Vdss $l.kg^{-1}$	3.82 ± 1.59	8.78 ± 8.77
Vd $l.kg^{-1}$	5.63 ± 3.11	10.02 ± 9.06
K10h^{-1}	0.081 ± 0.032	0.051 ± 0.017
K12h^{-1}	0.127 ± 0.080	0.060 ± 0.078
k21h^{-1}	0.071 ± 0.041	0.043 ± 0.021
Cl $ml.h^{-1}.kg^{-1}$	100.00 ± 20.00	80.00 ± 40.00

7.36 ± 1.40 and for milk 4.86 ± 1.90 μg/ml. Since, oxytetracycline has a bacteriostatic action, the drug concentration during the course of therapy should not fall below the minimum inhibitory concentration (MIC) in the mammary tissue of mastitic udder. In cows, Nouws *et al.*, (1985) reported that a mean maximum milk OTC concentrations in the range between 0.90 and 1.43 μg/ml, were achieved 12 to 24 h p.i. The OTC milk concentration-time profile ran parallel to the OTC plasma concentration-time profile. After intravenous administration the time for the appearance of OTC in milk was found to be shorter (1-2 hours p.i.), the peak milk OTC concentration was higher (1.7-1.9 μg/ml) and achieved earlier (6-8 h p.i.), and the OTC persistence in milk shorter than after i.m. administration. Mevius *et al*, (1986) reported that plasma OTC concentration exceeding 0.5 μg/ml was maintained for 48 h to 70 h and in milk for 33 to 49 hours. In cow calves MIC was maintained until 56 hours (Ziv, 1982), 48 hours (Luthman and Jacobsson, 1982; Josselin and Valentin-Smith, 1983) and in sheep until 56 hours (Ziv, 1982).

The pharmacokinetics analysis showed that following distribution equilibrium, the drug concentration versus time data of the elimination phase could be adequately described by two-compartment model kinetic analysis. The elimination kinetic parameters of the drug in plasma and milk are

presented in Table 2. Following absorption, the distribution equilibrium was attained during phase I which revealed a half-life value of 3.92 ± 3.06 hours for plasma and 7.57 ± 3.23 hours for milk. the distribution half-life in the present study wa shorter than that of conventional preparation 9.12 hours (Pulloud, 1973) and 20 hours (Luthman and Jacobasson, 1982). The elimination half-life (Phas II) was 38.25 ± 18.85 for plasma and 63.34 ± 64.13 for milk. The values of plasma half-life in mastitic buffaloes were almost three times longer than 13.6 hours in normal buffaloes (Verma and Paul, 1983). The longer half-life is attributable to slow release of the drug from the site of injection.

Total body clearance (Cl) represents the sum of metabolic and excretory processes and 100 ± 20ml.h^{-1}. kg^{-1} for plasma and 80 ± 40 ml.h^{-1} for milk. the major part of the drug was cleared through excretion into milk while the drug is mainly excreted unchanged through kidneys. Somatic cells count in the mastitic buffaloes at the start of experiment was 4.46 ± 0.73 millions/ml milk. Following intramuscular injection f Terramycin/LA 20 mg/kg 2.27 ± 0.41, 2.23 ± 0.26, 1.95 ± 0.18, 1.70 ± 0.23 and 1.33 ± 0.36 millions/ml milk. The count declined rapidly until day 3 after injection following which until day 19, the rate of decline was relatively slower. This decrease was related to the plasma and milk levels of the drug, which persisted above MIC level during the 7-8 days observation period. The results obtained in this study show that the Terramycin/LA provided significantly longer mean residence times of oxytetracycline both in plasma and milk than the conventional formulation and therefore single injection effectively decreased the somatic cells count in the buffaloes suffering from subclinical mastitis.

REFERENCES

Arret, B., D. P. Johnson and A. Kirshbaum, 1971. Outline of details for microbiological assays of antibiotics: second revision. J. Pharm. Sci., 60: 1689-1694.

Cornwell, K.L. 1980. Evaluation of long acting injectable oxytetracycline. Med. Vet. Pract., 61: 945-947.

Escudero, E., C. M. Carceles, and J. M. Serrano, 1994. Pharmacokinetics of oxytetracycline in goats: modifications induced by a long-acting formulation. Vet. Rec., 135(23): 548-552.

Jousselin, W. and A. Valentin-Smith, 1983. Long acting oxytetracycline for respiratory diseases of cattle, sheep and swine for preventing bovine chlamydial abortion. Vet. Pract. France, 67(7): 459-480.

Luthman, J. and S. O. Jacobsson, 1982. A comparison of two oxytetracycline formulations in cattle. Acta Vet. Scand., 23: 147-149.

Mevisu, D.J., J. F. Nouws, H. J. Breukink, T. B. Vree, F. Driessens and R. Verkaik, 1986. Comparative pharmacokinetics, bioavailability and renal clearance of five parenteral oxytetracycline-20% formulations in dairy cows. Vet. Q., 8(4): 285-294.

Nouws, J. F., H. J. Breukink, F. J. Binkhorst, J. Lohuis, P. van Lith, D. J. Mevius, and T. B. Vree, 1985. Comparative pharmacokinetics and bioavailability of eight parenteral oxytetracycline-10% formulations in dairy cows. Vet. Q., 7(4): 306-314.

Pulloud, M., 1973. Pharmacokinetics, plasma protein binding and dosage of oxytetracycline in cattle and horses. Rev. Vet. Sci., 15: 224-230.

Rashmussen, F., 1973. The mechanisms of drugs secretion into milk. In Dietary Lipids and Postnatal Development, Eds. c. Galli, A. Gocine & A. Pecile, Raven Press, NY., pp. 231-245.

Simpson, J. E. 1978. Sustained oxytetracycline blood concentration in swine due to unique long acting formulation. Proc. Int. Pig. Vet. Soc., 5th World Congress, Zagreb, p. 46.

Verma, K. J. and B. S. Paul, 1983. Pharmacokinetics and plasma protein binding (*in vitro*) of oxytetracycline in buffaloes (*Bos bubalis*). Am. J. Vet. Res., 44(3): 497-499.

Ziv, G. 1982. Serum oxytetracycline concentration in cows and calves after intramuscular injection of conventional and two long acting oxytetracycline preparations. Refuah Vet., 39(4): 154-160.