Effects of multiple injections of *Escherichia coli* endotoxin on the pharmacokinetics and dosage regimens of a long-acting formulation of oxytetracycline (OTC-LA) in cross-breed calves

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ABSTRACT

The effect of induced fever on the serum concentrations and pharmacokinetics of a long-acting formulation of oxytetracycline (OTC-LA) was studied in cross-bred calves. Fever was induced and maintained for 72 h by injecting *Escherichia coli* endotoxin (1µg/kg, i.v.) repeatedly at an interval of 12 h up to 48 h. In addition to producing a clear rise in temperature, endotoxin resulted in a significant ($P< 0.05$) increase in heart and respiration rates and other signs of clinical disease in calves. Subsequently, significant alterations were recorded in total leucocyte count (TLC), differential leucocyte count (DLC), blood glucose, blood urea nitrogen (BUN) and serum Zn, Cu, Fe and serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (AKP) concentrations in calves. Fever also produced significant perturbations in serum drug concentrations and reduction in absorption and elimination half-lives ($t_{1/2ka}$ and $t_{1/2cl}$), volume of distribution (Vd_{ss}) and time to reach peak concentration (T_{max}). Fever had no effect on peak serum drug concentration (C_{max}), area under curve (AUC), systemic clearance (Cl_B) and bioavailability (F) of drug. Calculations revealed that the dosage regimens of OTC-LA required to maintain a minimum serum drug concentration of 1µg/ml for 48 h in febrile calves would be 12.20 per cent (loading) and 21.82 per cent (maintenance) higher than required to produce identical serum concentrations of drug in healthy, afebrile calves.

Key words: pharmacokinetics, endotoxin, *E. coli*, fever, oxytetracycline, dosage regimens, calves

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Introduction

One of the major aims of conducting pharmacokinetic studies of antimicrobial agents is to formulate optimal dosage regimens that would be useful in the treatment of diseases caused by susceptible pathogens in animals. Most of these studies are, however, conducted on healthy animals and the pharmacokinetic data generated in them is extrapolated for use in diseased animals. Such extrapolation of pharmacokinetic data, of antimicrobial agents, does not seem scientifically valid, because several bacterial and blood protozoan infections and related diseases in which these drugs are used induce pathophysiological changes causing rise in body temperature and alterations in the basal metabolic rate in animal body (KUME and GARG, 1986). Studies have shown that diseases, particularly those which are associated with fever, markedly alter the pharmacokinetics of drugs in animals (VAN MIERT, 1990). These alterations in pharmacokinetic parameters during disease states may influence the efficacy of antibacterial therapy. To obtain the optimal efficacy of a drug it is necessary to modify its dosage regimens on the basis of pharmacokinetic data of the drug obtained during the actual disease state (KUMAR and MALIK, 1999a).

Oxytetracycline is a broad-spectrum antibiotic which is effective not only against both gram-positive and gram-negative bacteria but also against rickettsiae, mycoplasma, chlamydiae, and certain protozoa such as Balantidium, Anaplasma, and Amoeba (BYWATER, 1991). A long-acting formulation of oxytetracycline (OTC-LA) has recently been introduced in the Indian market to avoid repeated administration, thereby reducing the cost of treatment of bacterial infections caused by oxytetracycline sensitive microorganisms. Clinical trials have shown that a single intramuscular injection of this formulation was equivalent in efficacy to two daily administrations of a conventional formulation, when used for anaplasmosis (MAGONIGLE et al., 1978) or *Pasteurella* pneumonia (BREEZE and MAGONIGLE, 1979) and tropical bovine theileriosis (BAGHERWAL, 1989). In addition, a single injection of this formulation has been found to be effective in the therapy of bovine dermatophilosis (ILEMOBADE et al., 1979) and in the prevention of undifferentiated bovine respiratory tract disease occurring in feed lots (JANZEN and McMANUS, 1980).

An *Escherichia coli* endotoxin-based febrile model has been used extensively to study the effect of fever induced by gram-negative bacteria on the pharmacokinetics of antibacterial drugs including conventional formulation of oxytetracycline in animals (VAN MIERT, 1990), but no data are available regarding the effect of *E. coli* endotoxin-induced fever on OTC-LA in calves. The purpose of the present study was therefore to investigate (a) the effect of endotoxin (*E. coli*) induced fever on the serum concentrations and pharmacokinetics OTC-LA after intramuscular administration to cross-bred calves and (b) to derive satisfactory dosage regimens for intramuscular administration of OTC-LA for calves for use in febrile conditions.

Materials and methods

Animals. Nine healthy crossbred ruminating male calves, 4-6 months old and ranging in body mass from 70-80 kg, were used. The animals were procured from a college farm with no recent history of colibacillosis. The animals were kept indoors on straw bedding with free access to a mineral lick and water, and were fed hybrid Napier grass, hay and approximately 250 g of antibiotic-free pelleted feed twice daily. Each animal was quarantined for 2 weeks before start of experiment and was determined to be healthy by regular clinical examination. The study was carried out in the months of January and February, with average day temperatures of 18-22 °C.

Experimental disease model. Fever was induced by intravenous injections of *E. coli* lipopolysaccharide (LPS) endotoxin (Difco laboratories, Detroit, Michigan) in calves. Prior to administration, endotoxin was dissolved in sterile, pyrogen-free normal saline solution to make a stock solution containing LPS at concentration of 100 μg/ml body mass. The stock solution was further diluted to the required concentration (10μg/ml) and injected into jugular vein of animals at a dose of 1 μg/kg body mass. To produce fever of prolonged duration as encountered during naturally acquired colibacillosis, five such doses of freshly prepared LPS solution were administered to calves at an interval of 12 h up to 48 h. The doses required to produce a febrile response of this magnitude were

determined in another set of animals in our laboratory. The febrile response was found to be reproducible when repeated in both the groups of animals used in this study.

Experimental design. Animals were randomly divided into two groups. Animals of group A (n=4) were used to study the clinical and pathophysiological effects of fever on calves, while animals of group B (n=5) were used thrice at an interval of 14 days for studying the pharmacokinetics of long-acting formulation of oxytetracycline (OTC-LA) in calves. In phase 1, these animals were given OTC-LA by intravenous route. In phase II three animals received OTC-LA by deep intramuscular route. In last phase of experiment (Phase III) fever was induced in three animals prior to administration of OTC-LA by deep intramuscular route as in phase II of the experiment. All nine animals were subjected to clinical and physical examination, which did not include haematological and blood chemical analysis

Drug administration and sampling procedure. The long-acting formulation of oxytetracycline (OTC-LA) [Terramycin LA®; Pfizer India Ltd., Bombay] containing 200 mg of oxytetracycline dihydrate per ml of 2 pyrrolidine vehicle system was used. Drug was administered to animals by intravenous and/or intramuscular injection at a dose of 20 mg/kg body mass. The intramuscular injections of OTC-LA were made deep into gluteal muscle at two different sites after dividing the total dose into two equal parts, using a 21-guage needle. Blood samples (5 to 6 ml) were drawn from the left jugular venepuncture of group B animals (n=5) into sterile glass test tubes without anticoagulant at 0, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 24, 36 and 48 h, after intravenous and at 0, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 24, 36, 48, 60, 72 and 84 h, after intramuscular administration of OTC-LA. Blood samples from animals of group A were collected before (0 h) and at 1, 3, 6, 9, 12, 24, 48, 72 and 96 h after experimental fever for haemoglobin (Hb), packed cell volume (PCV), white blood cell (WBC) count, differential leucocyte count (DLC), blood glucose, blood urea nitrogen (BUN), serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase (AKP) and serum copper (Cu) iron (Fe) and Zinc (Zn) concentrations. Serum was collected by centrifugation ($1200 \times g$, 15 minutes at room temperature) within 2 h

of sample collection, and aliquot fractions were stored at –20 °C, until analyzed.

Haematological and blood chemistry analysis. The Hb, PCV, WBC counts and DLC were determined using methods described by JAIN (1986). Serum Cu, Fe and Zn concentrations were measured by atomic absorption/ flame spectrophotometer (model AA-646-V2, Shimadzu corporation, Kyoto, Japan) using a technique described earlier (KUMAR and MALIK, 1999b). Blood glucose, BUN and serum AST, ALT and AKP were estimated by methods described earlier (MUKHERJEE, 1988; WOOTON and FREEMAN, 1982)

Bioassay procedure. The serum OTC concentrations were determined using the agar gel diffusion method, (ARRET et al., 1971) using *Bacillus cereus* var. mycoides ATCC 11778 as test organisms. The minimum detection limit of assay was 0.125 µg/ml. The standard curve of OTC in calf serum was linear between 0.125 and 4 µg/ml. Pure oxytetracycline base for preparing standard curve was obtained from Cadila Laboratories Pvt. Ltd., Ahmedabad, Gujarat, India. The value of correlation coefficient (r) was more than 0.99. The repeatability of this assay was excellent and error within day estimation was less than 5 per cent.

Pharmacokinetic analysis. The disposition kinetics of OTC in each calf following intravenous administration was analyzed with the aid of a computer program for nonlinear regression analysis (YAMAOKA et al., 1981). The pharmacokinetic of OTC in calves were best described by a two compartment open model system. The program provided simultaneous estimations of various intercepts (A and B), hybrid constant (α and ß), distribution and elimination half-lives ($t_{1/2\alpha}$ and $t_{1/2\beta}$), area derived volume of distribution ($Vd_{(area)}$) and systemic clearance (Cl_B) of drug. The A, B, α and ß were used to calculate the rate constants K_{12} and K_{21}.

The volume of central compartment (V_C), the distribution volume at steady state (Vd_{ss}), area under zero moment of curve (AUC) and area under first moment of curve (AUMC) were calculated according to standard methods (GIBALDI and PERRIER, 1982). The intramuscular concentration data were analyzed with the aid of a computer program for non-compartment analysis based on the statistical moment theory (SMT) (YAMAOKA et al., 1978). The mean residence time (MRT) was calculated as MRT = AUMC/

AUC. The mean absorption time (MAT) was calculated as MAT = MRT_{im} - MRT_{iv}. The intercept B and the terminal elimination slope l_2 were calculated by linear least square regression analysis, using the last 6-8 serum concentrations vs. time data. Peak serum OTC concentration C_{max} and time to C_{max} (t_{max}) were read directly from the data. Bioavailability (F) was calculated using the method of corresponding areas as F=AUC_{im} X $dose_{iv}$ / AUC_{iv} X $dose_{im}$ x 100.

Dosage regimens. Dosage regimens for long-acting formulation for intramuscular administration in cow calves were computed using equations described earlier (KUMAR and MALIK, 1999a).

Statistical analysis. Significance of difference was tested with students paired t-test, or independent t-test, where appropriate. The null-hypothesis was rejected at the 5 per cent level. Values are presented as mean ± standard error from the mean (SE). Harmonic means ± pseudo-SE were calculated and used for all half-life values (LAM et al., 1985).

Results

Within ten minutes of the intravenous administration of endotoxin calves became dull and depressed. Soon after, the mean rectal temperature, heart and respiratory rates of these animals were significantly increased. Animals also showed slight difficulty in respiration. Auscultation of lung fields at this time revealed a few fine moist rales with decreased ventilation of peripheral fields. These changes are suggestive of pulmonary oedema. The mean body temperature remained >1 °C above normal from 2 to 72 h. The maximal rise in rectal temperature occurred between 5 to 7 h after each LPS challenge. Muscular fasciculations, ataxia and a desire to lie down were noticed in some calves. Three calves passed some loose faeces, but there was no diarrhoea. Various clinical signs of CNS depression including dullness, depression, muscular incoordination and anorexia were less marked on subsequent endotoxin administrations. Febrile response in calves (n=9) after repeated administration of *E. coli* endotoxin is depicted in Figure 1.

Haematological and biochemical changes. There were no significant changes in PCV and Hb concentration. However, significant leukopenia
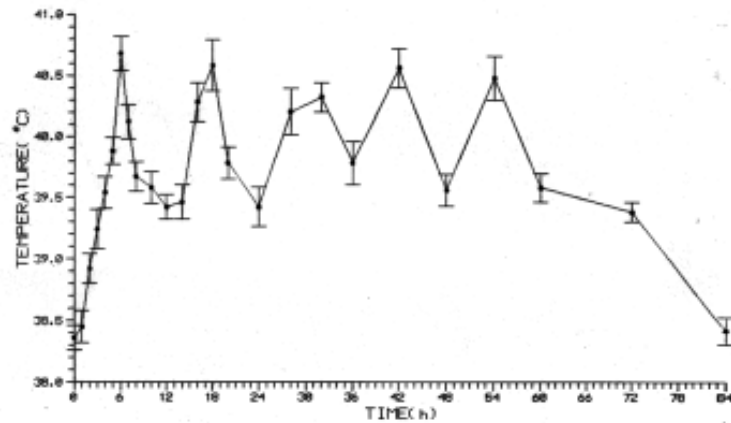
Fig.1. Mean rectal temperature (± SE) of nine calves just before and until 84 h after experimental fever with *E. coli* endotoxin

was observed between 6 and 9 h after endotoxin administration. Differential leucocyte count revealed significant neutrophilia with marked shift to immature cells. In addition, repeated dosing of endotoxin resulted in significant decrease in lymphocyte count between 6 and 96 h of LPS challenge (Table 1).

Table 1. Effect of multiple injections of *E. coli* endotoxin on haematological parameters in calves

Time (h)	Hb	PCV (%)	WBC (10³/ml)	Immature neutrophils (%)	Mature neutrophils (%)	Total neutrophils (%)	Ly (%)	Eo (%)	Mo (%)
0	10.20 ± 0.47	31.62 ± 1.25	5.50 ± 0.77	3.25 ± 0.48	18.50 ± 1.94	21.75 ± 1.80	75.50 ± 1.55	0.75 ± 0.25	1.75 ± 0.25
1	9.75 ± 0.56	28.62 ± 1.92	4.77 ± 0.65	4.25 ± 0.63	21.25 ± 1.02	25.50 ± 1.19	71.50 ± 1.26	1.25 ± 0.25	1.75 ± 0.25
3	8.95 ± 0.67	28.37 ± 1.65	2.89 ± 0.61	5.75 ± 0.85*	20.50 ± 2.72	26.25 ± 2.02	70.50 ± 2.10	1.25 ± 0.25	2.00 ± 0.41
6	9.35 ± 0.55	33.62 ± 1.28	2.00 ± 0.30**	10.75 ± 1.65**	27.75 ± 2.66*	38.00 ± 3.90**	58.75 ± 4.60*	1.25 ± 0.25	2.00 ± 0.71
9	9.95 ± 0.51	33.50 ± 1.75	2.80 ± 0.18*	17.25 ± 1.25**	32.50 ± 4.94*	49.75 ± 4.80**	47.00 ± 5.15**	1.00 ± 0.00	2.25 ± 0.75
12	9.96 ± 0.53	34.25 ± 2.62	3.74 ± 0.57	22.50 ± 2.10**	12.80 ± 0.82*	33.70 ± 2.40**	61.25 ± 2.80**	1.50 ± 0.29	2.75 ± 0.48
24	9.80 ± 0.29	32.62 ± 1.43	4.64 ± 0.78	28.00 ± 3.70**	9.80 ± 0.91**	36.20 ± 3.37**	59.50 ± 3.77**	1.25 ± 0.25	2.25 ± 0.25
48	9.35 ± 0.26	31.62 ± 0.90	4.85 ± 0.76	18.00 ± 3.24**	21.00 ± 3.24	39.00 ± 1.78**	57.75 ± 2.46**	1.25 ± 0.25	2.00 ± 0.58
72	9.60 ± 0.22	29.62 ± 0.94	4.24 ± 0.81	15.75 ± 1.93**	19.75 ± 2.78	35.50 ± 1.19**	62.50 ± 2.00**	1.25 ± 0.25	1.75 ± 0.63
96	9.75 ± 0.26	30.36 ± 0.99	4.36 ± 0.87	13.00 ± 2.04**	19.25 ± 4.27	32.25 ± 2.18**	64.50 ± 2.72*	1.00 ± 0.00	2.00 ± 0.58

* $P < 0.05$; ** $P < 0.01$

The changes in serum zinc, iron, and copper are shown in Table 2. Significant reductions in serum concentration in Cu, Fe, and Zn were observed from 3 h onward from endotoxin challenge. Serum zinc concentration returned to normal after 24 h, and that of serum Cu concentration by 72 h after endotoxin administration, while those of serum iron remained depressed until the end of experiment (Table 2).

Table 2. Effect of multiple injections of *E. coli* endotoxin on serum enzyme, trace metals, blood glucose and blood urea nitrogen in calves

Time (h)	AST (μmol/min/L)	ALT (μmol/min/L)	AKP (KAU/100 ml)	Zinc (μg/100 ml)	Iron (μg/100 ml)	Copper (μg/100 ml)	Glucose (mg/100 ml)	BUN (mg/100 ml)
0	26.00±2.10	18.34±0.57	9.18±2.45	162.62±2.97	224.75±4.90	79.95±1.80	55.28±1.80	21.12±2.20
1	28.83±3.20	26.52±1.76**	8.10±1.48	155.87±2.68	214.36±3.94	75.00±1.76	62.10±1.58**	19.38±2.30
3	36.43±2.30*	21.23±3.32	12.80±2.27	127.37±3.02**	190.87±4.84**	66.12±1.66**	55.12±4.88	18.57±2.76
6	26.39±3.82	20.54±2.76	12.52±1.98	83.25±2.56**	158.37±1.69**	59.00±1.74**	45.38±3.50**	20.50±3.02
9	28.90±4.66	19.50±3.02	10.46±1.70	68.87±2.76**	133.00±2.67**	67.87±2.43*	43.53±4.03**	21.43±3.25
12	33.02±7.23	24.00±2.22	9.28±2.03	109.75±2.76**	116.12±3.04**	72.86±2.15*	42.38±2.80*	22.24±3.72
24	32.00±4.63	21.64±2.57	9.10±2.22	126.12±2.62**	104.25±2.55**	61.12±2.18**	50.30±2.18	18.72±2.42
48	32.04±3.59	19.76±4.02	8.03±1.43	139.12±2.26	119.12±1.96**	57.00±2.40**	46.16±3.80	17.56±2.16
72	19.26±3.02	17.90±4.50	7.55±1.58	156.50±1.64	143.87±2.00**	70.12±2.62*	51.42±4.08	15.44±1.87
96	28.06±3.08	21.63±3.30	8.56±2.30	167.25±1.16	168.00±2.15**	75.00±2.26	53.22±2.22	14.28±1.67*

* P<0.05; ** P<0.01; AST = aspartate aminotransferase; ALT = alanine aminotransferase; AKP = alkaline phosphatase

E. coli lipopolysaccharide induced a biphasic response in blood glucose concentrations in calves. There was a significant increase in blood glucose concentration at 1 h followed by a significant decrease in its concentration from 6 to 12 h post endotoxin challenge. Conversely, BUN showed a marked decline from 48 h onwards and was significantly (P<0.05) low even at 96 h after endotoxin administration. There was a significant (P<0.05) increase in serum AST and ALT at 3 and 1 h after endotoxin administration, whereas serum levels of alkaline phosphatase remained unchanged throughout the experiment.

Pharmacokinetics. Mean serum concentration of OTC vs time data for a single intravenous dose of 20 mg/kg OTC-LA in five calves is shown in Figure 2. After i.v. injection the mean distribution and elimination half-lives were 0.39 and 9.58 h, the mean V_c $Vd_{(area)}$ and Vd_{ss} were 0.213, 1.056 and 0.866 l/kg, respectively, whereas mean total body clearance of OTC

was 74.66 ± 3.23 ml/h/kg. Mean serum drug concentrations >1.0 µg/kg were maintained for 36 h (Figure 2).

Mean serum OTC concentration vs time data for a single intramuscular administration of 20 mg/kg OTC-LA in calves before and after repeated
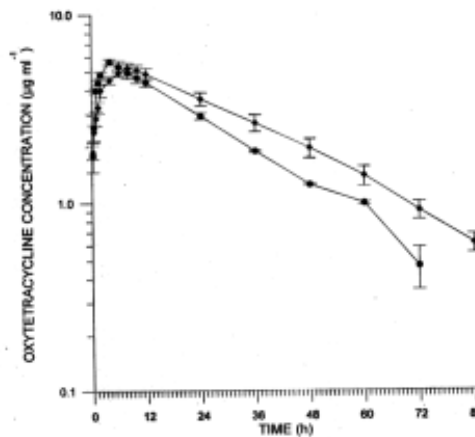

Fig. 2. Mean serum concentration vs time data (± SE) of oxytetracycline
following intravenous administration of long-acting formulation of oxytetracycline
20 mg/kg body mass to five calves

endotoxin administration is presented in Figure 3. Fever caused a significant decline in serum OTC concentrations at 1, 1.5, 4 h and from 24 to 72 h after drug administration. No drug could be detected in the serum of febrile calves 72 h after drug administration. Fever which resulted in significant reduction in absorption and elimination half-lives ($t_{1/2ka}$ and $t_{1/2el}$), and volume of distribution of drug without affecting its C_{max} values. Serum t_{max} values, however, decreased significantly from 8.40 h to 4.40 h in febrile calves. No significant differences were observed in the values of AUC Cl_B and bioavailability (F) of drug between healthy and febrile animals (Table 4).

Table 3. Pharmacokinetic parameters of oxytetracycline after intravenous injection of
20mg/kg body mass of a long-acting formulation of oxytetracycline to five calves

Pharmacokinetic values	Unit	Mean ± SE
C_s	μg/ml	94.96 ± 3.75
A	μg/ml	79.18 ± 3.38
B	μg/ml	15.28 ± 1.42
$t_{½\,α}$	h (arithmetic)	0.40 ± 0.04
$t_{½\,α}$	h (harmonic)	0.39 ± 0.04
$t_{½\,β}$	h (arithmetic)	9.83 ± 0.72
$t_{½\,β}$	h (harmonic)	9.58 ± 1.00
MRT	h	11.64 ± 0.56
K_{12}	h^{-1}	1.127 ± 0.108
K_{21}	h^{-1}	0.349 ± 0.044
Kcl	h^{-1}	0.352 ± 0.020
AUC	μg h/ml	270.00 ± 12.22
V_C	L/kg	0.213 ± 0.009
$Vd_{(area)}$	L/kg	1.056 ± 0.084
Vd_{ss}	L/kg	0.866 ± 0.043
Cl_B	ml/h/kg	74.66 ± 3.23

Dosage regimens. Taking 24 and 48 h as convenient dosage intervals
(t), with minimum therapeutic concentration C^{min}) of 0.5, 1, 2, 3, 4, 6 and
8 μg/ml, and using values of ß and Vd_{ss} of Table 2, the dosage regimens of
long-acting formulation of oxytetracycline for intramuscular administration
were computed for both healthy and febrile calves and are presented in
Table 5. Calculations revealed that the dosage regimen of OTC-LA required
to maintain minimum serum drug concentration of 1.0 μg/ml for 48 h in
febrile calves would be 12.20 per cent (loading dose) and 21.82 per cent
(maintenance dose) higher than required to produce identical serum
concentrations of the drug in healthy afebrile animals.

Table 4. Effect of experimental fever on the pharmacokinetics of oxytetracycline in
calves after intramuscular injection of 20 mg/kg body mass of a long-acting
formulation of oxytetracycline to five calves

| Pharmacokinetic values	Unit	Afebrile animals (Mean ± SE)	Febrile animals (Mean ± SE)
$t_{1/2\,ka}$	h (arithmetic)	17.78 ± 1.47	13.34 ± 0.56*
$t_{1/2\,ka}$	h (harmonic)	17.60 ± 1.47	13.24 ± 0.66*
MAT	h	25.67 ± 2.12	19.25 ± 0.80*
C_{max}	µg/L	5.34 ± 0.31	5.77 ± 0.29
t_{max}	h	8.40 ± 0.40	4.40 ± 0.40**
$T_{1/2\,el}$	h (arithmetic)	25.85 ± 1.24	21.40 ± 0.33**
$T_{1/2\,el}$	h (harmonic)	25.63 ± 1.26	21.38 ± 0.36*
MRT	h	37.30 ± 1.79	30.88 ± 0.48**
AUC	µg h/ml	236.63 ± 19.08	191.44 ± 9.83
Vd_{ss}	L/kg	2.78 ± 0.15	2.31 ± 0.13*
Cl_B	ml/h/kg	74.65 ± 3.23	74.62 ± 3.20
F	per cent	87.50 ± 5.46	71.70 ± 5.69

* $P<0.05$; ** $P<0.01$
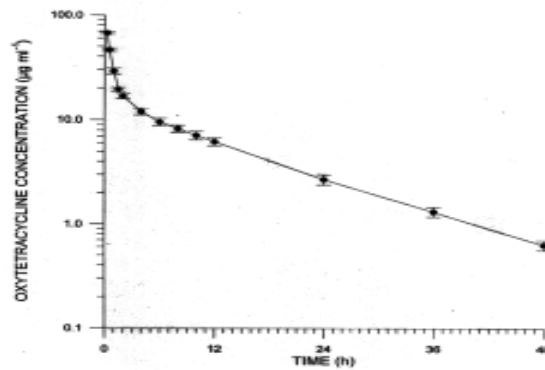
Fig. 3. Mean serum concentration vs time data (± SE) of oxytetracycline after
experimental fever with *E. coli* endotoxin following intramuscular administration of
long-acting formulation of oxytetracycline, 20 mg/kg body mass to five calves

Table 5. Calculated intramuscular dosage regimen of long-acting formulation
of oxytetracycline for calves

Desired serum concentration (μg/ml)	Dosing interval (h)	Afebrile calves		Febrile calves	
		Priming dose (mg/kg)	Maintenance dose (mg/kg)	Priming dose (mg/kg)	Maintenance dose (mg/kg)
0.5	24	2.18 ± 0.16	1.04 ± 0.08	2.35 ± 0.11	1.27 ± 0.06
	48	2.87 ± 0.22	2.08 ± 0.17	3.22 ± 0.15	2.54 ± 0.12
1.0	24	4.36 ± 0.32	2.09 ± 0.17	4.70 ± 0.22	2.54 ± 0.11
	48	5.74 ± 0.43	4.17 ± 0.34	6.44 ± 0.30	5.08 ± 0.23
2.0	24	8.72 ± 0.64	4.18 ± 0.34	9.40 ± 0.45	5.08 ± 0.22
	48	11.48 ± 0.86	8.35 ± 0.68	12.88± 0.60	10.16 ± 0.45
3.0	24	13.08 ± 0.96	6.26 ± 0.52	14.10 ± 0.66	7.62 ± 0.34
	48	17.22 ± 1.29	12.50 ± 1.02	19.32 ± 0.89	15.24 ± 0.68
4.0	24	17.44 ± 1.28	8.36 ± 0.68	18.80 ± 0.90	10.16 ± 0.45
	48	22.96 ± 1.72	16.68 ± 1.36	25.76 ± 1.20	20.40 ± 0.92
6.0	24	26.16 ± 1.92	12.54 ± 1.02	38.20 ± 1.35	15.24 ± 0.68
	48	34.44 ± 2.58	25.00 ± 2.04	41.47 ± 1.79	30.48 ± 1.38
8.0	24	34.88 ± 2.56	16.70 ± 1.36	37.60 ± 1.80	20.32 ± 0.90
	48	45.92 ± 3.44	33.36 ± 2.72	51.52 ± 2.38	40.64 ± 1.84

Discussion

Febrile disease model. The result of the present study clearly
demonstrated that repeated intravenous administration of *E. coli* endotoxin
is capable of inducing a prolonged fever in crossbred calves. This is in
contrast to earlier reports that repeated injections of *E. coli* endotoxin lead
to development of tolerance to endotoxin with decreased intensity and
duration of febrile response in animals (BENNETT and BEESON, 1950).
Furthermore, the sustained febrile response observed in calves used for
studying the patho-physiological effects of endotoxin was reproducible,
as an almost similar febrile response was observed in endotoxin treated
calves employed in the pharmacokinetic study of oxytetracycline. Besides
cattle (WRAY and THOMLINSON, 1972; MUSA et al., 1972) other animal species,
including buffalo calves (MODY, 1989), goats (KUME and GARG, 1986), and
sheep (WILSON et al., 1984) have also been reported to respond to *E. coli*
endotoxin in a similar way. Previous studies have shown that certain
endogenous pyrogens, collectively known as interleukins, are released from
activated phagocytic cells and reticulo-endothelial cells of the host in
response to inflammation, tissue injuries, bacterial toxins and other
diseases. Once released into circulation they act on the thermoregulatory
centre of hypothalamus through PGE_2 and raise hypothalamic thermostat
to febrile levels (VAN MIERT, 1990). The increase in respiration and heart

rate observed in the present study is similar to those reported in goats (VAN MIERT et al., 1983), calves (WRAY and THOMLINSON, 1972; REECE and WAHLSTROM, 1973) and sheep (BLATTEIS et al., 1988). Hypotension induced by *E. coli* endotoxin has been held responsible for tachycardia observed in calves, dogs and goats (REECE and WAHLSTROM, 1973; SWAN and JACOBSON, 1967; VAN MIERT and VAN DUIN, 1979). As in the present study, Hb and PCV remained unaffected on exposure to *E. coli* LPS (GRIEL et al., 1975). The alterations in neutrophil count and total leucocyte count are similar to those reported earlier in cattle given multiple injections of *E. coli* (TEMPLETON et al., 1988) and buffalo calves given graded doses of this endotoxin (JAIN et al., 1989). Elevated serum concentrations of AST and ALT are indicative of tissue damage, while reduction in serum levels of Zn, Fe and Cu might be due to their redistribution to the reticulo-endothelial system, as part of a non-specific defence mechanism of the organism to bacterial toxins (VAN MIERT, 1990). Similar perturbations in serum levels of these trace elements have been reported in endotoxin-treated buffalo calves (MODY, 1989) and goats (VAN MIERT et al., 1983). The alterations in blood glucose concentrations were related to the release of catecholamines and corticosteroids in the initial stages of *E. coli* endotoxin administration in calves, and those in BUN to increased renal clearance in the later stages of the experiment. A similar decline in BUN has been previously reported in goats given low doses of staphylococcal enterotoxin B and F (VAN MIERT et al., 1983). Elevated serum concentrations of AST and ALT are indicative of tissue damage.

Pharmacokinetics. Fever-induced marked alterations in the pharmacokinetics of OTC are evidenced by significantly lower values of $t_{\frac{1}{2}ka}$, MAT, t_{max}, Vd_{ss}, $t_{\frac{1}{2}el}$ and MRT of OTC obtained from febrile calves as compared to values obtained from the same animals before induction of fever. The shorter absorption half-life and MAT values in febrile animals appears to be due to faster absorption of drugs from their site of deposition. It is probable that increased cardiac output and blood flow to the muscles prompted the drug molecules to move faster from their site of deposition to the blood circulation, resulting in shorter MAT, $t_{\frac{1}{2}ka}$ and t_{max} value of OTC during fever. Previous reports have indicated that during rising fever, blood flow in sheep and goats shifts away from the heat loss tissues (e.g.

skin) to heat production tissues (VAN MIERT et al., 1983), whereas cardiac output increases (BLATTEIS et al., 1988). During rising fever, induced with *E. coli* endotoxin, the rate of absorption of ampicillin from shivering muscles was faster, resulting in a significantly higher serum concentration of the antibiotic than in the control afebrile goats (GROOTHUIS et al., 1980). In contrast, the absorption rate of ampicillin from non shivering muscles (e.g. neck muscles) was slower, resulting in significantly lower serum concentrations of antibiotic than in control afebrile veal calves (GROOTHUIS et al., 1978).

Distribution of a drug is a complex phenomenon and depends on the physiochemical interactions between drug molecules and the body. Patho-physiological changes, such as alteration in capillary blood flow, tissue perfusion, drug protein binding, tissue pH, and fluid compartment shifts are known to affect the distribution space of a drug in a diseased animal (KLOTZ, 1976; VAN MIERT, 1990). Circulatory depression leading to delayed attainment of pseudodistribution equilibrium of drug or reduction in the extra-cellular compartment volume under influence of endotoxin could be one of the factors responsible for reduced space for OTC in calves. Moreover, physical stress due to endotoxin can alter body hydration and subsequently the distribution space of drugs (VAN MIERT, 1990). Alterations in endocrine functions in endotoxin-treated animals, particularly that of thyroid function, are also known to alter the distribution of drugs in tissues (BLATTEIS et al., 1988; VAN MIERT, 1990). Although temperature related changes in binding to serum proteins have been demonstrated for several drugs (BALLARD, 1974), this mechanism is probably not applicable to OTC, which is not highly bound to serum proteins. Reduction in the OTC distribution volume and half-life recorded in the present study is similar to that recorded earlier in endotoxin-treated febrile rats (BERGERON and BERGERON, 1986) and horses (WILSON et al., 1983). As the changes in OTC distribution and elimination constants occurred in opposite directions, total serum clearance remained unaffected by the endotoxin-induced fever. Given the dependence of half-life (or the elimination rate constant) on both clearance and volume of distribution, the changes in the latter could effect the changes in half-life, clearance remaining constant, without altering the overall rate of drug elimination (ROWLAND, 1978). The chances of OTC undergoing increased

biotransformation in febrile calves is less likely as it is a water soluble drug and is excreted mainly via kidney and, to some extent, in bile as an intact drug (NOUWS et al., 1985). The alterations in the haematological and biochemical parameters observed following multiple injections of *E. coli* endotoxin, are, as expected, part of acute phase response only (VAN MIERT, 1990) and probably do not contribute significantly to the changes in various pharmacokinetic parameters of OTC in calves. It was therefore concluded that the total body clearance of OTC was not affected by fever and that changes in the $t_{1/2el}$ reflected change in the volume of distribution.

The ultimate objective of the present study was to calculate satisfactory dosage regimens of oxytetracycline that can be used effectively in clinical practice for the treatment of mild to severe bacterial infections causing fever in cattle. The MIC for tetracycline of most susceptible pathogenic bacteria in cattle (*Bacillus anthracis, Corynebacterium pyogenes, Listeria monocytogenes, Mycoplasma* spp., *Pasteurella* spp., *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae*) range between 0.12 and 1μg/ml (SCHIFFERLI et al., 1982). Given 50% protein binding and a safety factor of 2-4, the minimum serum therapeutic concentrations (C^{min}) of OTC for most pathogenic bacteria of cattle would be in the range of 0.5-8 μg/ml (KUMAR and MALIK, 1998).

To maintain minimum serum therapeutic concentrations of drug during disease condition, which significantly alters pharmacokinetic behaviour, either the dose or its frequency has to be altered. Since alterations in the frequency of the drug administration could lead to managemental problems, the doses were computed keeping the dosing interval constant. It is anticipated that the proposed dosage regimens will serve as useful guidelines for the effective treatment of febrile diseases caused by oxytetracycline sensitive gram positive and gram- negative bacteria, in cattle. The final proof of effective serum drug concentration of an antibacterial agent, however, rests in its clinical effectiveness. Moreover, oxytetracycline has a post-antibiotic residual inhibitory effect (PAE) against *E. coli* and a similar but shorter effect against methicillin-resistant *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* (ZHANEL and CRAIG, 1994). The duration of PAE depends on the concentration of drug and duration of exposure. It has been demonstrated

that maximum PAE values occur at concentrations between 5- and 10-fold greater than MIC, suggesting that serum drug concentration may be allowed to fall below the MIC between peak levels achieved by repeated administration of the drug (KORITZ, 1984; POWERS et al., 1984; ZHANEL and CRAIG, 1994). The microorganisms that have been previously treated with supra inhibitory concentrations of antibiotic and presently in PAE phase, are very sensitive to the sub- inhibitory concentrations of antibacterial concentrations of the antibacterial agent (ZHANEL and CRAIG, 1994). Therefore, exposure of bacteria in the PAE phase to sub-inhibitory concentrations may not only delay growth for several hours, but may even be bacterial. Considering these effects, it would be desirable to suitably prolong the dosage intervals of OTC in clinical circumstances.

It is concluded that endotoxin (*E. coli*) induced prolonged fever markedly influences the pharmacokinetics of OTC-LA in calves, and to obtain optimal therapeutic effect the clinician may have to alter the dosage regimen of the drug to treat microbial infections caused by OCT-sensitive microorganisms under field conditions. However, the present study gives no information on appropriate OTC concentrations at the site of infection. This aspect needs further classification.

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**KUMAR, R., J. K. MALIK: Učinci višestrukog uštrcavanja endotoksina
bakterije *E. coli* na farmakokinetiku i doziranje oksitetraciklina s produženim
djelovanjem u teladi križanih pasmina. Vet. arhiv 71, 245-263, 2001.**

SAŽETAK

U ovom istraživanju potaknuta je i održavana vrućica u tijeku 72 sata ponovljenim uštrcavanjem
endotoksina bakterije *E. coli* u razdoblju od 12 pa sve do 48 sati. Osim naglog nastupa vrućice,
ubrizgavanjem endotoksina postignut je i značajan porast bila kao i učestalost disanja te drugi znakovi
bolesti u goveda. Povrh toga, učinak se očitovao i u promjenama u krvnoj slici, i to u odnosu na
ukupan broj leukocita, diferencijalnu krvnu sliku, glukozu u krvi, mokraćevinu, te razinu cinka, bakra,
željeza, zatim serumske aspartat-aminotransferaze, alanin-aminotransferaze i alkalne fosfataze. Vrućica
je poremetila i serumske koncentracije lijeka te uzrokovala i smanjenu apsorpciju, poluživot raspada
i volumensku distribuciju kao i vrijeme potrebno za postizanje najviše koncentracije. Vrućica nije
utjecala na najvišu serumsku koncentraciju, područje ispod krivulje, sustavnu eliminaciju te biološku
raspoloživost lijeka. Istraživanja su pokazala da je za isti učinak lijeka u goveda s vrućicom potrebna
veća količina lijeka da se postigne serumska koncentracija od 1 μg/ml tijekom 48 sati, odnosno
12,20% za početan učinak te 21,82% za održavanje u odnosu na onu potrebnu za zdrava goveda.

Ključne riječi: farmakokinetika, endotoksin, *E. coli*, vrućica, oksitetraciklin, doziranje, telad