Pharmacokinetic Profile of Erythromycin after Intramammary Administration in Lactating Dairy Cows with Specific Mastitis

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ABSTRACT

The pharmacokinetics of erythromycin was studied in five lactating dairy cows following single intramammary infusion of 300 mg erythromycin in each of two quarters per cow with specific mastitis. Levels of erythromycin in plasma and quarter milk samples were measured by agar plate diffusion assay using *Micrococcus luteus* (ATCC 9341) as the test organism. Erythromycin level in plasma reached a peak concentration value (C_{max}) of $0.07 \pm 0.01 \mu g/ml$ at 30 min; thereafter, levels declined gradually to reach $0.05 \pm 0.00 \mu g/ml$ 12 h post drug administration. The pharmacokinetic profile of the drug revealed mean absorption half life ($t_{1/2ka}$) as 0.26 ± 0.05 h. The drug was eliminated slowly with elimination half-life ($t_{1/2\beta}$) of 13.75 ± 0.35 h and elimination rate constant (k_{el}) of $0.04 \pm 0.00 h^{-1}$. The volume of distribution based on the zero-time plasma concentration intercept of the least-squares regression line of the elimination phase ($V_{d(B)}$) was 0.032 L/kg. The drug crossed to untreated quarters also; mean drug levels of 0.20 ± 0.07, 0.23 ± 0.07, 0.17 ± 0.04, and $0.17 \pm 0.04 \mu g/ml$ were found at 3, 6, 8 and 12 h, respectively. The mean drug concentration for treated quarters was measured as $22.97 \pm 2.31 \mu g/ml$ milk at first milking (12 h) following drug infusion. No apparent adverse reaction was seen in cows administered erythromycin. It is concluded that following intramammary infusion erythromycin diffuses readily and extensively in various body fluids and tissues and adequate concentration is maintained in udder tissues for at least 12 h post intramammary administration. Thus, erythromycin may be recommended for local therapy of acute mastitis caused by Gram-positive bacteria in lactating dairy cows.

Keywords: cows, erythromycin, intramammary, pharmacokinetics, specific mastitis

Abbreviations: ATCC, American Type Culture Collection; $AUC_{0-\infty}$, area under the curve from zero to infinity by the trapezoidal integral; $AUMC_{0-\infty}$, area under the moment curve; β, elimination rate constant; C_0, zero-time plasma drug concentration intercept of the least-squares regression line of elimination phase; C_{max}, peak plasma drug concentration value; IDF: International Dairy Federation; k_a, absorption rate constant; k_{el}, overall elimination rate constant; MIC: minimum inhibitory concentration; MRT, mean residual time; $t_{1/2ka}$, absorption half life; $t_{1/2\beta}$, elimination half-life; $V_{d(B)}$, volume of distribution based on zero time plasma drug concentration intercept of the least-squares regression line of elimination phase

INTRODUCTION

Mastitis, the inflammatory process of the udder, is caused by a variety of infectious agents and is a common reason for antibiotic therapy in lactating dairy cows. The majority of spontaneous cases of mastitis in lactating dairy cows are caused by Gram-positive bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis*, *Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis* and *Corynebacterium bovis* (Radostits *et al.*, 2000). Therefore, for intramammary therapy during lactation, antibiotics with good efficacy against these Gram-positive organisms are preferred (Cattell *et al.*, 2001). A large

number of intramammary preparations containing different antibiotics are available for use in mastitis therapy, but many of them have limited practical utility for reasons such as high cost, development of antibiotic resistance, low clinical efficacy (Owens *et al*., 1997), long milk withdrawal period (Smith *et al*., 2004) and persistence of antibiotic residues in milk even after recommended withdrawal periods (Seymour *et al*., 1988). Erythromycin is a low-cost macrolide antibiotic having good distribution in mammary gland after intramammary administration (McKeller, 1991). It is lipophilic in nature with high activity at alkaline pH (Hardman *et al.,* 2001). Owing to the high alkalinie activity and lipophilia, erythromycin is expected to readily cross the blood–mammary gland barrier and reach effective levels in milk after parenteral administration (Ballarini, 2001). The drug has shown marked *in vitro* activity against a variety of organisms isolated from milk of dairy cows with mastitis (Owens *et al*., 1990; Malinowski *et al*., 1992; Watts and Salmon, 1997). Recent clinical trials have also shown beneficial effects of erythromycin therapy in spontaneous cases of bovine mastitis, particularly those caused by Gram-positive bacteria (Shephard *et al*., 2000; Bajwa *et al.,* 2005). Although a few studies are available on the pharmacokinetic profile of erythromycin following its parenteral administration in healthy animals, there seems to be no report in this regard after its intramammary infusion in diseased or healthy quarters of lactating cows. The present study was undertaken to determine the pharmacokinetic profile and disposition pattern of erythromycin in plasma and milk of treated and untreated quarters after intramammary administration of the drug in lactating dairy cows with specific mastitis.

MATERIALS AND METHODS

Animals

The study was conducted at an organized cattle farm, where the animals were kept under semi-loose housing system with identical feeding and management practices. Holstein-Friesian × Sahiwal crossbred lactating cows not administered with any antibiotic for at least the previous 21 days, and below 3 months of lactation, were screened for udder health status. The individual quarter foremilk samples from each animal were collected and subjected, for three consecutive days, to somatic cell count analysis (Fossomatic method) and culture isolation by standard microbial procedures of the National Mastitis Council (Brown *et al.,* 1981). On the basis of somatic cell count and culture results of milk samples for three consecutive days, the udder quarters were classified into healthy, latent infection, non-specific mastitis and specific mastitis according to the criteria of the International Dairy Federation (1987) (Table I).

Five cows representing similar udder health status, i.e. each having two quarters affected with specific mastitis (foremilk somatic cell count greater than 500×10^3 cells/ml and culturally positive for udder pathogen) and two healthy functional quarters (foremilk somatic cell count usually less than 200×10^3 cells/ml and culturally negative) were selected and included in the present study. The pathogens isolated from the mastitic quarters of the five cows comprised *Staphylococcus aureus* (6 quarters) and *Streptococcus agalactiae* (4 quarters). The animals were 5–7 years of age (2nd to 3rd calving) with a body weight of 400–450 kg. All the cows were in early lactation (45–80 days in milk) with a production

TABLE I
Criteria for classification of udder quarters into healthy or mastitis groups

Somatic cell count (cells/ml milk)	Udder pathogen	
	Not detected (⩾2 times)	Detected (⩾2 times)
All three times <500 000	Healthy	Latent infection
At least one time >500 000	Non-specific mastitis	Specific mastitis

level of 11–12 litres of milk per cow per day. The cows were machine milked twice a day at 12 h milking interval.

Drug administration

The cows selected were isolated from the rest of the herd and put into individual pens. Baseline blood and milk samples were collected at routine morning milking and each of the two quarters with specific mastitis was infused with a commercial intramammary preparation containing erythromycin I.P. 300 mg ("Bovimast" from Indo-Biocare Pvt. Ltd., Vadodara, India).

Sample collection

From each cow a blood sample of about 20 ml was collected into separate heparinized vials by jugular venepuncture once before, and 15 min, 30 min, and 1, 2, 3, 4, 6, 8 and 12 h after drug infusion. The samples were immediately brought to the laboratory and centrifuged at 200 g for 15 min for plasma separation. The collection of individual quarter milk samples (about 30 ml) was done at 3, 6, 8 and 12 h from untreated quarters, and at 12 h from treated quarters. No blood and milk samples were collected thereafter, but the animals were infused with drug up to five subsequent milking for treatment of mastitis quarters. The milk and plasma samples were stored at −20°C and analysed for drug levels within 2 weeks of sample collection.

Drug assay

The levels of erythromycin in plasma and milk samples were estimated following the method of Arret and colleagues (1971) using *Micrococcus luteus* (ATCC 9341) as test organism. The seed culture of the test organism was procured from Institute of Microbial Technology, Chandigarh, India. Using a punching device, six wells of equal diameter were punched at equal distance in each assay plate containing seeded Antibiotic Medium No. 11. Three assay plates were used for each sample. Three alternate wells on each plate were filled with erythromycin standard solution (reference concentration 1 μg/ml) and the remaining three wells with test samples (either plasma or milk). Before actual analysis, the milk samples

were appropriately diluted with 0.1 mol/L phosphate buffer (pH 8) to bring the drug levels within detection range of the assay. However, it was not necessary to dilute the plasma samples at any stage. The plates were incubated at 35°C for 16–18 h and the mean inhibition zone diameter was measured and corrected for nine replicates of each sample, taking into consideration the zone of inhibition of erythromycin standard solution. The concentration of erythromycin in plasma and milk samples was calculated from the respective standard curve. A standard curve for erythromycin was prepared in pooled antibiotic-free calf plasma for assay of drug in blood plasma and in 0.1 mol/L phosphate buffer (pH 8) for analysis of drug in milk samples. The standard curves for plasma and milk were linear between 0.05 and 1.5 μg/ml erythromycin, with a mean correlation coefficient (r^2) of standard curves >0.98 for plasma and >0.99 for buffer. The minimal detectable concentration of the assay both for plasma and milk was 0.05 μg/ml.

Pharmacokinetic analysis

The plasma drug concentration data obtained were analysed for study of the kinetics of the drug. The slope of the terminal phase of elimination phase (λ) was calculated as $\lambda = 2.303 \times m$, where m is regression coefficient obtained by the least-squares technique. Zero-time plasma drug concentration (C_0) was calculated as antilog C. The value of C was calculated from the equation of the line $y = m\bar{x} + c$. Taking x as zero and substituting the values of y, x and m, the value of C was calculated as $C = y - m\bar{x}$. The area under the plasma concentration–time curve ($AUC_{0-\infty}$) and area under the first moment curve ($AUMC_{0-\infty}$) were calculated by the trapezoidal rule for all measured data, with extrapolation to infinity using C_L/λ, where C_L is the plasma drug concentration at last sampling and λ is the slope of the terminal phase of elimination. The mean residence time (MRT) and overall elimination rate constant (k_{el}) were calculated as $AUMC_{0-\infty}/AUC_{0-\infty}$ and 1/MRT, respectively. The $0.693/k_a$ represented the absorption half-life ($t_{1/2k_a}$), and $0.693/\beta$ The elimination half-life ($t_{1/2\beta}$). The volume of distribution based on zero-time plasma drug concentration intercept of the elimination phase ($V_{d(B)}$) was found from X_0/C_0, where X_0 is the dose of drug/ kg body weight and C_0 is the zero-time plasma drug concentration intercept of the least-squares regression line of the elimination phase.

Statistical analysis

Values obtained were expressed as mean $\pm$ SE. The standard errors were calculated from mean data according to Snedecor and Cocharan (1989).

RESULTS AND DISCUSSION

No adverse mammary gland reaction was observed in any cow following intramammary infusion of erythromycin. The plasma concentration–time profile of erythromycin is presented graphically in Figure 1. The mean plasma erythromycin concentration 15 min after intramammary drug administration was 0.06 ± 0.01 μg/ml. The peak plasma concentration value (C_{max}) of 0.07 ± 0.01 μg/ml was reached 30 min after drug administration. This level was
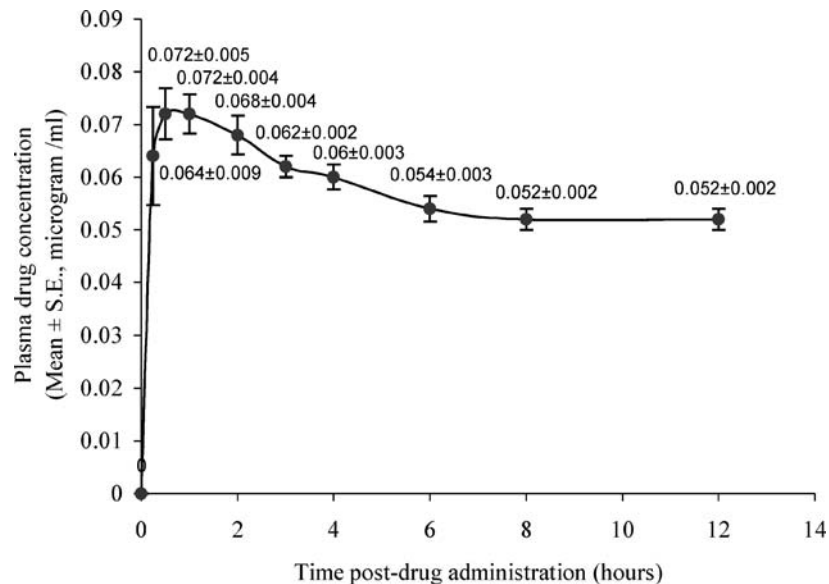
Figure 1. Plasma erythromycin concentrations (mean ± SE) following intramammary administration of drug in specific mastitic quarters of lactating dairy cows ($n = 5$)

maintained up to 1 h and thereafter declined gradually to reach a level of $0.05 \pm 0.00\,\mu g/ml$ at 12 h post drug administration. The rapid appearance of erythromycin in plasma suggests that the drug was rapidly absorbed and entered the systemic circulation following intramammary administration. The rate of absorption of different chemotherapeutic agents after intramammary administration varies depending upon their dissociation constant (pK_a) and lipid solubility. For example, oxacillin is absorbed twice as rapidly as benzylpenicillin (Ziv and Sulman, 1974). Oxacillin is absorbed quickly and appears in blood serum as early as 10 min post administration (Bansal *et al.*, 2003). On the other hand, cefacetrile sodium cannot be detected in blood plasma even after 10 h following single intramammary infusion of 235 mg of cefacetrile sodium per quarter in lactating dairy cows (Carli *et al.*, 1983). Erythromycin is rapidly absorbed following intramuscular or subcutaneous administration in cattle (Burrows *et al.,* 1989). However, to our knowledge no report is available with respect to erythromycin on the degree of systemic absorption following intramammary administration for comparison with the findings of the present study.

The inspection of plasma erythromycin concentration–time curves indicated that the drug followed a one-compartment open model. The pharmacokinetic analysis of the drug is summarized in Table II. The total area under the plasma drug-concentration–time curve (AUC) and the total area under the first moment of the plasma drug concentration–time curve (AUMC) was $12.84 \pm 0.85\,(\mu g.h)/ml$ and $300.02 \pm 31.76\,(\mu g.h^2)/ml$, respectively. The mean value for volume of distribution based on zero-time plasma drug concentration intercept of the elimination phase ($V_{d(B)}$) was $0.032\,L/kg$. The volume of distribution based on total area under the plasma drug concentration time curve ($V_{d(area)}$) after intravenous administration of drug in calves was $1.59\,L/kg$ (Burrows *et al.*, 1989). This clearly indicated

TABLE II

Pharmacokinetic profile of erythromycin following single intramammary administration of 300 mg of erythromycin in each of two mastitic quarters per dairy cow

Pharmacokinetic parameter	Unit	Cow number					Mean $\pm$ SE
		I	II	III	IV	V	
C_0	µg/ml	46.99	48.30	58.86	48.30	45.90	49.67 $\pm$ 2.34
$AUC_{0-\infty}$	(µg.h)/ml	13.54	15.46	10.32	11.97	12.93	12.84 $\pm$ 0.85
$AUMC_{0-\infty}$	(µg.h^2)/ml	324.83	399.71	207.87	266.44	301.24	300.02 $\pm$ 31.76
MRT	h	23.99	25.86	20.14	22.26	23.31	23.11 $\pm$ 0.95
$t_{1/2k_a}$	h	0.17	0.22	0.24	0.24	0.45	0.26 $\pm$ 0.05
$t_{1/2\beta}$	h	13.97	14.44	13.22	14.44	12.67	13.75 $\pm$ 0.35
k_{el}	h^{-1}	0.04	0.04	0.05	0.04	0.04	0.04 $\pm$ 0.00
$V_{d(B)}$	L/kg	0.032	0.031	0.029	0.033	0.033	0.032 $\pm$ 0.00

extensive penetration and resultant high concentration of erythromycin in various body fluids and tissues. It was seen that following intramammary administration, the drug was absorbed rapidly ($t_{1/2k_a}$ 0.26 $\pm$ 0.05 h) and eliminated slowly ($t_{1/2\beta}$ 13.75 $\pm$ 0.35 h). The elimination half-life recorded in the present study is close to the value of 11.85 h reported in calves after its intramuscular administration by Burrows and colleagues (1989). They further observed that half-life increased with dose, suggesting that disposition of erythromycin followed dose-dependent kinetics. Half-life also varied with route of administration and was as high as 26.87 h following subcutaneous administration (Burrows *et al.*, 1989). The rate of elimination of erythromycin from the central compartment (k_{el}) was 0.04 $\pm$ 0.00 h^{-1}, with mean residence time (MRT) of 23.11 $\pm$ 0.95 h, suggesting that the drug was retained longer in the systemic circulation.

Drug concentration levels in milk samples at different time intervals for treated and untreated quarters are presented in Tables III and IV, respectively. The concentration of erythromycin in milk samples of treated quarters 12 h after intramammary infusion varied from 15 to 30 µg/ml, with a mean value of 22.97 $\pm$ 2.31 µg/ml. The drug crossed to untreated quarters, and mean concentration levels of 0.20 $\pm$ 0.07, 0.23 $\pm$ 0.07, 0.17 $\pm$ 0.04 and 0.17 $\pm$ 0.04 µg/ml were detected at 3, 6, 8 and 12 h post infusion, respectively. The MIC of erythromycin for common mastitis pathogens is estimated to be 0.5 µg/ml (Salmon *et al.*, 1998). Thus, the therapeutic concentration of erythromycin is maintained in infused quarters for at least 12 h. The results of the present study are supported by the work of Wuschko and colleagues (1998), who following intracisternal injection of 300 mg erythromycin observed therapeutically effective concentrations of drug in milk up to 24 h post infusion.

Thus, observation of various pharmacokinetic parameters reveals that erythromycin is absorbed rapidly, is extensively distributed in various body fluids and tissues, and is cleared slowly after its intramammary administration in quarters with specific mastitis. The drug may be used as an intramammary preparation in the therapy of bovine mastitis caused by Gram-positive bacteria.

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TABLE III
Concentration of erythromycin in treated quarter milk samples 12 h after single intramammary infusion of 300 mg erythromycin in each of two quarters with specific mastitis per dairy cow

Cow number	Quarter (treated)	Milk drug concentration (µg/ml)
I	LF	30.00
	LH	30.00
II	LF	21.70
	LH	17.50
III	LH	15.00
	RH	17.50
IV	RF	15.00
	LH	[a]
V	RF	30.00
	RH	30.00
Mean ± SE	–	22.97 ± 2.31

[a]Sample could not be analysed

TABLE IV
Concentration of erythromycin in two untreated quarter milk samples at different time intervals following single intramammary infusion of 300 mg erythromycin in each of two other quarters with specific mastitis per dairy cow

Cow number	Quarter (untreated)	Milk drug concentration (µg/ml) at time interval			
		3 h	6 h	8 h	12 h
I	RF	[a]	0.06	0.06	0.08
	RH	[a]	0.05	0.5	0.08
II	RF	[a]	0.06	0.06	0.08
	RH	[a]	0.06	0.06	0.08
III	LF	0.06	0.25	0.20	0.30
	RF	0.10	0.30	0.25	0.40
IV	LF	0.05	0.08	0.08	0.25
	RH	0.25	0.62	0.2	0.08
V	LF	0.50	0.25	0.2	0.08
	LH	0.25	0.62	0.1	0.25
Mean ± SE	–	0.20 ± 0.07	0.23 ± 0.07	0.17 ± 0.04	0.17 ± 0.04

[a]No detectable concentration found

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