Pharmacokinetics and penetration of danofloxacin from the blood into the milk of cows

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Shem-Tov, M., Rav-On, O., Ziv, G., Glickman, A., Saran, A. Pharmacokinetics and penetration of danofloxcacin from the blood into the milk of cows. *J. vet. Pharmacol. Therap.* **21,** 209–213.

The single-dose disposition kinetics of danofloxacin were determined in clinically normal lactating cows after intravenous (i.v.) and intramuscular (i.m.) administration of the drug at 1.25 mg/kg. The drug concentrations in blood serum and milk were determined by microbiological assay methods and the data were subjected to kinetic analysis. The mean i.v. and i.m. elimination half-lives ($t_{½el}$) in serum were 54.9 and 135.7 min, respectively. The steady-state volume of distribution (V_{ss}) was 2.04 L/kg. The drug was quickly absorbed after i.m. injection but a 'flip flop' effect was clearly evident and bioavailability was > 100%. Penetration of danofloxacin from blood into milk was rapid and extensive with drug concentrations in milk exceeding those in serum beginning 90–120 min after i.v. and i.m. administration and onwards. Milk danofloxacin concentrations equal to or higher than the minimal inhibitory concentrations (MIC) for pathogenic Gram-negative bacteria and *Mycoplasma* species were maintained over ≈24 h.

Concentrations greater than the MIC for *Staphylococcus aureus* were maintained in the milk for 12 h.

(Paper received 19 October 1997; accepted for publication 15 December 1997)

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INTRODUCTION

Danofloxacin is an antibacterial fluoroquinolone developed for exclusive use in animals. The drug possesses good *in vitro* activity against a variety of pathogens, including Gram-positive and Gram-negative bacteria and *Mycoplasma.* The rapid bactericidal activity of danofloxacin is the result of its inhibition of bacterial DNA gyrase (Neer, 1988). In several ways danofloxacin is very similar to other fluoroquinolones such as enrofloxacin, ciprofloxacin and marbofloxacin (Van Custen *et al.*, 1990; Brown, 1996); they share a wide spectrum of antimicrobial activity, a large volume of distribution and are active at low concentrations.

The pharmacokinetics of antibiotics, including danofloxacin, may change in lactating animals (Oukessou *et al.*, 1990; Petracca *et al.*, 1993; Soback *et al.*, 1994). The plasma-to-milk drug concentration ratio is often unknown and in human patients safety considerations during maternal drug intake lead to recommended suspension of breast feeding (Atkinson & Begg, 1990). This ratio may be of other considerations in lactating cows. The pharmacokinetic properties of danofloxacin were evaluated in young calves and adult cattle (Grimshaw *et al.*, 1990; Giles *et al.*, 1991; Apley & Upson, 1993; Friis, 1993). The aim of the present study was to determine the pharmacokinetic parameters and penetration of danofloxacin from blood into the milk in lactating cows following i.v. and i.m. treatment.

MATERIALS AND METHODS

Animals

Studies were conducted on 14 clinically normal Israeli-Holstein cows of variable age; they were toward the end of their lactation and produced 18–26 L of milk/day. Their udders secreted macroscopically normal milk, of pH 6.6–6.9, with somatic cell counts of < 500 000/mL. Cows were located at the Volcani Agricultural Institute dairy herd, Bet-Dagan, Israel; they were housed in open shade corrals, had free access to drinking water and were fed antibiotic free total mixed ration. (The main compositions of the total mixed ration were 17% proteins and 1.7 Mcal net energy/kg DM).

Experimental design

Advocin® Injectable Solution, lot # 505000 (Pfizer Inc, Istanbul, Turkey) was used. It was injected intravenously ($n = 7$ cows) into the right jugular vein and intramuscularly ($n = 7$ cows) into the lower third of the right side of the neck at 1.25 mg/kg body weight. The treatments were given 15–30 min after the last milking of lactation and the cows were not milked regularly after treatment.

Jugular vein blood samples, of ≈ 5 mL each, were collected from the left side at 10, 20, 30, 40, 50, 60, 80, 100, 120, 150, 180, 240, 360, 480 and 600 min and also 24 h after i.v. dosing.

Blood samples were collected at 15, 30, 60, 90, 120, 180, 240, 360, 480 and 600 min and also 24 h after i.m. treatment. The blood was allowed to clot at room temperature (22 °C) for 2–3 h. Then the serum was separated by centrifugation ($1000 \times \boldsymbol{g}$) and was stored at −20 °C together with dilutions of spiked danofloxacin standards prepared on the day of treatment in antibiotic-naive pooled bovine serum. Quarter milk samples were collected, by stripping each gland as completely as possible, within 5 min of each blood sampling period. The milk samples, 3–5 mL each, were placed at −20 °C within 30 min of collection along with dilutions of danofloxacin standards prepared in antibiotic-naive pooled bovine milk.

Danofloxacin assay

The concentrations of danofloxacin in the serum and milk were determined by an agar plate diffusion method (Bennett *et al.*, 1966) using *E. coli* ATCC 25922 as assay microorganism growing on Mueller-Hinton agar (Difco Laboratories, Detroit, MI, USA). This assay does not distinguish between danofloxacin and its microbiologically active metabolites. Therefore, the results are expressed as serum and milk danofloxacin antimicrobial equivalent activity. To simplify the presentation, however, the term 'concentration' is used throughout the text.

Six wells, 8.0 mm in diameter, were cut at equal distances into a standard (100 mm) plastic Petri plate containing 12 mL of the seeded agar. The wells were filled with 50 μL of either the test samples or danofloxacin standards. The plates were kept at room temperature for 4 h before incubation at 37°C for 14–16 h.

The inhibition zone diameters were measured and the danofloxacin concentrations in the test samples were calculated from the standard curve. Semilogarithmic plots of the inhibition zone diameter vs. standard danofloxacin concentrations in serum and milk ranging between 0.04 μg/mL and 5.0 μg/mL were linear with typical correlation coefficients > 0.967 for serum and > 0.981 for milk, (for the standard curves). The limits of quantitation in serum and milk were determined to be 0.02 and 0.04 μg/mL, respectively, based on diameter of inhibition zones in the range of $> 9.5 - < 30$ mm. The intra day assay accuracy and precision in serum were determined using five replicates of two-fold dilutions of drug standard solutions at concentrations ranging between 0.04 and 5.0 μg/mL. A similar procedure was applied to milk. The assay accuracy for serum was 91% for 0.1 μg/mL, and 104% for 5.0 μg/mL; precision was $< 4\%$. The assay accuracy for milk was between 90% for 0.1 μg/mL and 107% for 5.0 μg/mL; precision was $< 7.5\%$. Interday assay accuracy was determined on 6 separate days using danofloxacin dilutions in serum and milk covering the range of the assay. The interday assay accuracy for milk and serum was nearly the same, i.e. between 95% and 105%, while precision was $< 5\%$. Recovery of danofloxacin from serum and milk over the concentration range was 90–96%.

Pharmacokinetic analysis

The disposition kinetics of danofloxacin in each animal following i.v. treatment was analysed from the unweighed concentrations using the computer program ESTRIP (Brown & Manno, 1978). A two-compartment open model system (Baggot, 1977) was found to best fit the data. The distribution and elimination half-lives ($t_{1/2}$ a and $t_{1/2}$ b), the volume of distribution at steady-state (V_{ss}) and the total body clearance (Cl_B) were computed according to standard equations (Gibaldi & Perrier, 1982).

The i.m. serum concentration data, as well as the milk data, were analysed by both compartmental and non compartmental methods based on the statistical moment theory (SMT) (Yamaoka *et al.*, 1978) using an appropriate computer program (Yamaoka, 1986). The mean residence time (MRT) was calculated as MRT = *AUMC*/*AUC*, where *AUC* is the area under the concentration vs. time curve to infinity and *AUMC* is the area under the curve of the product of time and drug concentration vs. time from zero to infinity. Peak serum and milk danofloxacin concentrations (C_{max}) and time of C_{max} (T_{max}) were read directly from the data. The mean absorption time (MAT) was calculated as $MRT_{i.m.}$–$MRT_{i.v.}$ and bioavailability (F) was calculated as $AUC_{i.m.}/AUC_{i.v.} \times 100$. Only the mean MAT and F-values are presented as a crossover treatment design was not used.

The penetration of danofloxacin from the blood into the milk after i.v. and i.m. treatments was calculated and expressed as $C_{max\text{-}milk}/C_{max\text{-}serum}$, AUC_{milk}/AUC_{serum} and $t_{1/2el\text{-}milk}/t_{1/2el\text{-}serum}$. Kinetic values for each quarter of the udder were calculated separately. The results are presented as mean ± SD. The nonparametric Mann–Whitney test was used for comparing mean pharmacokinetic values in serum and milk after i.v. and i.m. drug administration; P-values < 0.05 were considered significantly different.

RESULTS

Danofloxacin administration to the lactating cows did not produce any adverse clinical signs or side-effects. Mean serum and milk concentration–time curves for danofloxacin after i.v. and i.m. administration are presented in Fig. 1. Pharmacokinetic values are summarized in Table 1. Danofloxacin concentrations in the serum decreased in a bi-exponential manner after i.v. administration, indicating the presence of distribution and elimination phases and justifying the use of a two-compartment kinetic model for analysing the data. The distribution and elimination half-lives were 11.4 ± 2.7 min and 54.9 ± 11.4 min, respectively, and the V_{ss} was 2.04 ± 1.10 L/kg. As indicated in Fig. 1 and Table 1, danofloxacin was rapidly absorbed after i.m. administration; the T_{max} was at 60 min, the $t_{1/2abs}$ was 31.6 ± 7.2 min but the mean MAT was 305.9 min. The drug was eliminated from serum after i.m. treatment at a significantly slower rate than after i.v. treatment, suggesting the presence of a 'flip-flop' effect in lactating cows. Consequently, F, the intramuscular availability, calculated by the method of corresponding areas, resulted in a very large mean value of 289.0% (Table 1).

Danofloxacin penetrated rapidly and extensively from blood into milk after both i.v. and i.m. treatments. In milk, drug

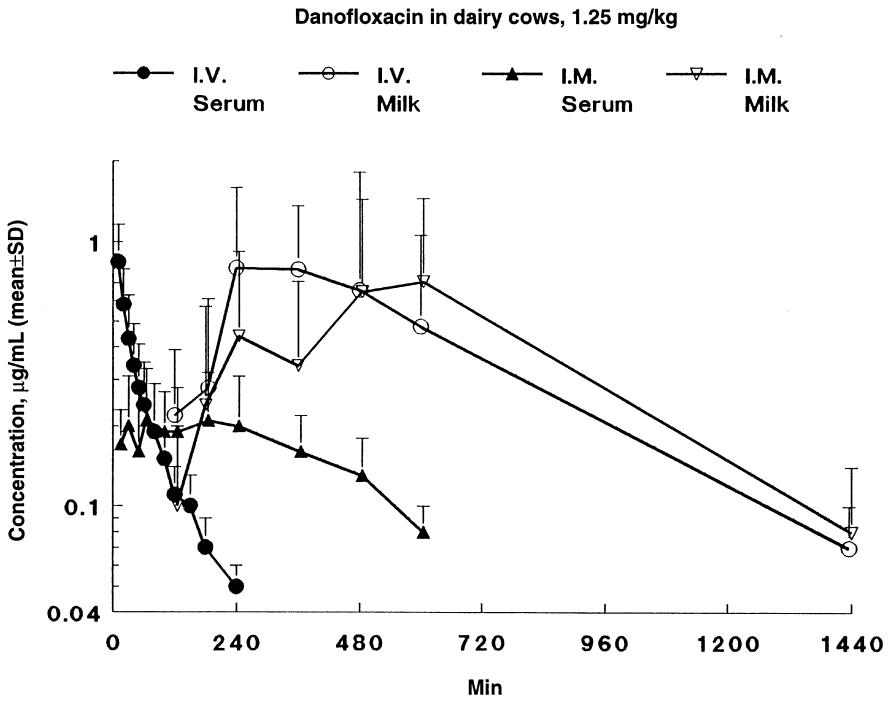

Fig. 1. Concentrations of danofloxacin in serum and milk of lactating cows after i.v. and i.m. administration of danofloxacin at 1.25 mg/kg. (●) – serum after i.v., (○) – milk after i.v., (▲) – serum after i.m., (▽) – milk after i.m.

Table 1. Pharmacokinetic values for danofloxacin injected intravenously and intramuscularly to lactating cows at 1.25 mg/kg

		Route of administration			
Pharmacokinetic value	unit	Intravenous Mean ± SD		Intramuscular Mean ± SD	
Serum					
Cp°	µg/mL	1.21	0.62		
C_{max}	µg/mL			0.28	0.09
T_{max}	min			60.00	0.00
A	µg/mL	0.86	0.43		
B	µg/mL	0.35	0.18		
$t_{1/2}\alpha$	min	11.42	2.73		
$t_{1/2}\beta$	min	54.86	11.39		
MRT	min	75.29	25.12	381.17	39.81
$t_{1/2absr}$	min			31.60	7.20
$t_{1/2el}$	min			135.70	53.10
MAT	min			305.88	
V_{ss}	L/kg	2.04	1.10		
AUC	µg/mL·min	45.89	19.55	132.85	30.66
Cl_B	mL·min/kg	0.03	0.03		
F	% of i.v. dose			289.00	
Milk					
C_{max}	µg/mL	0.80	0.79	0.71	0.74
T_{max}	min	240.00	0.00	600.00	0.00
$t_{1/2el}$	min	155.70	21.60	171.30	81.00
AUC	µg/mL·min	283.00	125.70	468.00	217.40

Cp° = Peak drug concentration after i.v. treatment, C_{max} = Peak drug concentration, T_{max} = Time of C_{max}, A = Zero-time intercept of the distribution phase, B = Zero-time intercept of the elimination phase, $t_{1/2}\alpha$ = Distribution phase half-life, $t_{1/2}\beta$ = Elimination phase half-life, MRT = Mean residence time, $t_{1/2absr}$ = Absorption half-life, $t_{1/2el}$ = Elimination half-life, MAT = Mean absorption time, V_{ss} = Steady-state volume of distribution, *AUC* = Area under the concentration vs. time curve, Cl_B = Total body clearance, *F* = intramuscular availability.

concentrations 90 min after i.v. injection, and 120 min after i.m. injection were equal to those in serum and were considerably higher than those in the serum thereafter (Fig. 1). At 3 and 4 h after i.v. administration mean concentrations of danofloxacin in milk were 4 and 15 times greater than the corresponding serum drug concentrations. At 4 and 6 h after i.m. treatment mean concentrations of danofloxacin in milk were 2.3 and 5.5 greater than the corresponding concentrations in the serum. Whereas the drug was not detected in the serum 6 h after i.v. treatment and 12 h after i.m. injection, mean drug concentrations in the milk at 24 h after i.v. and i.m. administration were > 0.10 µg/mL (Fig. 1). It is obvious from Fig. 1 that the drug apparently persisted for a longer time in the milk than in the serum. The calculated ratio $C_{max\text{-}milk}/C_{max\text{-}serum}$ after i.m. treatment was 2.54, the ratios AUC_{milk}-to-AUC_{serum} after i.v. and i.m. treatments were 6.16 and 3.52, respectively, and the ratios $t_{1/2el\text{-}milk}$-to-$t_{1/2el\text{-}serum}$ after i.v. and i.m. treatments were 2.84 and 1.26, respectively.

DISCUSSION

The pharmacokinetic interpretation of the serum danofloxacin concentration data reveals that the distribution of the drug in the body of lactating cows is very large. This is similar to its kinetic behaviour in non lactating cattle (Grimshaw *et al.*, 1990; Giles *et al.*, 1991; Apley & Upson, 1993; Friis, 1993) and is true of some other fluoroquinolones (norfloxacin, enrofloxacin and marbofloxacin) in ruminants (Soback *et al.*, 1994; Brown, 1996; Shem-Tov *et al.*, 1997). Experimental data are also available on the distribution of danofloxacin in the respiratory tract of pigs. For example, the mean steady-state bronchial secretion-to-serum, bronchial mucosa-to-serum, lung tissue-to-serum and

bronchial lymph nodes-to-serum concentrations ratios were 1.22, 3.68, 4.77 and 5.84, respectively (Friis, 1994).

Interpretation of the data gathered in the course of the present study also must take into consideration the assay method used (microbiological) and the sensitivity of the assay method. The antimicrobial fluoroquinolones represent a class of drugs known to be metabolized to a variable extent by different species (Green & Budsberg, 1993; Hooper & Wolfson, 1993; Brown, 1996). Although a good agreement was shown between microbiological and chemical (HPLC) test results for several fluoroquinolones, notably norfloxacin in pigs (Shem-Tov *et al.*, 1994) and enrofloxacin in cows (Walser *et al.*, 1993), it will be mere speculation to extend these latter observations to danofloxacin in cows. Because the ratio of the parent drug to its metabolites may not remain constant through the dosing interval, and the movement of the metabolites from blood into the milk may not be the same as for the parent drug, interpretation of the concentrations derived from the microbiological assay for the purpose of establishing minimally effective concentrations in serum and milk becomes confounded.

An additional confounding factor may be the presence of a 'deep' body compartment for danofloxacin in cows, as was suggested for several fluoroquinolones (Brown, 1996). The characteristics of a 'deep' compartment can not be revealed unless the limit of drug quantitation is very low. The sensitivity of the assay method used in the present study did not facilitate determination of drug concentrations in serum samples collected 6 h and 12 h after i.v. and i.m. drug treatment, respectively (Fig. 1). Thus, the possible presence of a third, slower phase of drug elimination from serum, which may be similar to the rate of decline in drug concentration in the milk, could not be explored. Although this latter issue may not have any relevance to therapy, it might be of importance when the residue of the drug is of interest.

It is generally accepted that xenobiotics cross the blood–milk barrier in the udder by non ionic passive-diffusion and the extent of diffusion is greatly influenced by the physicochemical properties of the drug (Rasmussen, 1966; Atkinson & Begg, 1990). Because of the presence of a carboxylic acid and one or more basic amine-functional groups, danofloxacin, like several other fluoroquinolones, is amphoteric and considered zwitterionic. However, between the pKa of the acidic and basic functional groups, between pH 6 and 8, these compounds are sufficiently lipid-soluble to be able to penetrate tissues (Brown, 1996). The milk in the mammary gland at any given time may be considered a peripheral body compartment, particularly when a considerable amount of the drug can be found in the milk (Atkinson & Begg, 1990). The extensive penetration of danofloxacin from blood into the cow's milk of pH 6.6–6.9 was, therefore predictable on the basis of the 'ion trap' mechanism (Rasmussen, 1966; Atkinson & Begg, 1990). Due to the greater persistence of drug concentrations in the milk relative to those in serum, the drug accumulated in the milk presenting $t_{1/2el\text{-}milk}$-to-$t_{1/2el\text{-}serum}$ ratios after i.v. and i.m. treatments of 2.84 and 1.26, respectively, and AUC_{milk}-to-AUC_{serum} ratios after i.v. and i.m. treatments of 6.16 and 3.52, respectively. Our observations on the extensive penetration of danofloxacin from the blood into the milk of lactating cows supported earlier findings on the pharmacokinetics of other fluoroquinolones, such as enrofloxacin in lactating cows (Franklin & Astrom, 1986; Walser *et al.*, 1993; Kaartinen *et al.*, 1996; Malbe *et al.*, 1996), norfloxacin in lactating ewes (Soback *et al.*, 1994), marbofloxacin in the milk of sows (Petracca *et al.*, 1993) and marbofloxacin in the milk of lactating cows and ewes (Shem-Tov *et al.*, 1998).

Several reports indicate that the serum $t_{1/2el}$ of some fluoroquinolones may be shorter in lactating than non lactating animals of the same species. The i.v. $t_{1/2\beta}$ of enrofloxacin in cattle was reported as 5.5 ± 0.9 h (Sheer, 1987) whereas the corresponding value in lactating cows was 1.7 h (Walser *et al.*, 1993)and 1.68 ± 0.18 h in another study (Kaartinen *et al.*, 1996). The $t_{1/2el}$ of marbofloxacin in pregnant sows was significantly shorter than in lactating sows (Petracca *et al.*, 1993). In lactating cows, the i.v. $t_{1/2\beta}$ of marbofloxacin was 2.07 ± 0.66 h (Shem-Tov *et al.*, 1998) whereas in non lactating adult cattle the corresponding value was 5.72 ± 1.17 h (Thomas *et al.*, 1994). Similar findings were reported (Soback *et al.*, 1994) after i.v. administration of norfloxacin to lactating and non lactating ewes. Admittedly, $t_{1/2}$ is not a good parameter for discussing the comparative rate of fluoroquinolone elimination in lactating vs. non lactating animals because $t_{1/2}$ is a hybrid parameter which depends on both clearance and volume of distribution. However, the latter values, particularly after i.m. administration, are not always available from the literature cited. In the present study the mean half-lives of 54.8 min and 135.7 min after i.v. and i.m. administration are considerably shorter than the 3.5–4.5 h values reported for danofloxacin after i.v., i.m. and subcutaneous administration to non lactating cattle, and assayed by HPLC (Grimshaw *et al.*, 1990; Giles *et al.*, 1991). Thus, despite reservations due to different assay methods and pharmacokinetic analysis procedures, comparative studies strongly suggest that lactation may increase the rate of elimination of some fluoroquinolones from the serum. It is restated that cows were treated immediately after the last milking just before dry-off and were not milked after treatment.

The minimal inhibitory concentrations (MIC) of danofloxacin for 90% of the field isolates of *Mycoplasma* species was 0.008–0.5 μg/mL (Cooper *et al.*, 1993) whereas for Gram-negative and Gram-positive udder pathogens the values are 0.05–2.5 μg/mL and 0.25–5.0 μg/mL, respectively (Ziv, unpublished). Danofloxacin concentrations equal to or higher than the MIC for Gram-negative bacteria and *Mycoplasma* species were maintained in the milk of cows for 12–24 h after a single i.v. and i.m. injection at the manufacturer's recommended dose of 1.25 mg/kg. This dose produced, for at least 12 h, milk danofloxacin concentrations higher than the MIC for *Staphylococcus aureus.* These findings suggest a potential use of danofloxacin in the treatment of bovine mastitis. It should be recalled that fluoroquinolones are concentration dependent antimicrobial agents (Hooper & Wolfson, 1993) and obtaining a high peak milk concentration is likely to be just as relevant to therapy as maintaining a MIC over the dosage interval.

The clinical and bacteriological efficacy of systemic danofloxacin treatment of bovine mastitis can best be determined from the

results of properly designed, well controlled field efficacy studies or experimental infections (Shukken & Deluyker, 1995). Results of the present investigation suggested that for the design of such studies a pivotal dose of 1.25 mg/kg q 24 h is justified for treatment of mastitis associated with Gram-negative pathogens and 1.25 mg/kg q 12 h for treatment of mastitis caused by *S. aureus*.

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