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Pharmacokinetic Disposition of Subcutaneously Administered Enrofloxacin in Goats

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ABSTRACT

The pharmacokinetic disposition of enrofloxacin was studied in goats after subcutaneous (s.c.) administration at a single dose of 7.5 mg/kg body weight. Blood samples were drawn from a jugular vein into heparinized tubes at predetermined time intervals after administration of the drug and the plasma was separated by centrifugation. The concentrations of enrofloxacin in the plasma were determined by a microbiological assay using *Escherichia coli* as the test organism. The plasma concentration–time data were analysed by non-compartmental methods. Enrofloxacin was rapidly absorbed, an appreciable concentration of the drug (0.30 ± 0.13 μg/ml) being present in the plasma by 5 min after s.c. administration. The maximum plasma concentration of enrofloxacin and the time to reach that maximum were 2.91 ± 0.39 μg/ml and 2.9 ± 0.51 h, respectively. A detectable concentration of enrofloxacin persisted in the plasma for 12 h. The elimination half-life and mean residence time of enrofloxacin were 2.84 ± 0.57 and 5.74 ± 0.28 h, respectively. It is suggested that enrofloxacin given subcutaneously may be useful in the treatment of susceptible bacterial infections in goats.

Keywords: enrofloxacin, fluorinated quinolones, goats, pharmacokinetics, subcutaneous injection

Abbreviations: $AUC_{0-\infty}$, area under the plasma concentration–time curve; $AUMC_{0-\infty}$, area under the first moment curve; β, slope of elimination phase; C_{max}, maximum plasma drug concentration; Cl_B/F, total body clearance divided by the fraction absorbed; MIC, minimum inhibitory concentration; MRT, mean residence time; s.c., subcutaneous; $t_{\frac{1}{2}\beta}$, elimination half-life; t_{max}, time to reach maximum plasma concentration; V_{dss}/F, apparent volume of distribution at steady state divided by the fraction absorbed

INTRODUCTION

Enrofloxacin is a fluorinated quinolone carboxylic acid derivative developed exclusively for use in animals (Altreuther, 1987). The drug has high antimicrobial activity against a broad spectrum of Gram-negative and Gram-positive bacteria and mycoplasmas, its minimum inhibitory concentration (MIC) values being in the range 0.001 to 1 μg/ml for many veterinary pathogens (Prescott and Yielding, 1990). Enrofloxacin is partly de-ethylated to an antimicrobially active metabolite, ciprofloxacin. The pharmacokinetic characteristics of enrofloxacin have been determined in cattle, pigs, dogs, sheep and horses (Küng *et al.*, 1993; Mengozzi *et al.*, 1996; Kaartinen *et al.*, 1997a; Stegemann *et al.*, 1997; Anadon *et al.*, 1999). The potent antimicrobial and

favourable pharmacokinetic properties of enrofloxacin, including its high bioavailability and excellent tissue penetration in different animal species, indicate that the use of this broad-spectrum fluoroquinolone antimicrobial agent is appropriate in goats for the treatment of infections caused by susceptible organisms (Brown, 1996). The subcutaneous route has been shown to be appropriate for the administration of enrofloxacin in calves, cattle and camels (Gavrielli *et al.*, 1995; Kaartinen *et al.*, 1995; Martinez-Larranaga *et al.*, 1997). However, to the best of our knowledge, the subcutaneous disposition kinetics of enrofloxacin have not been reported in goats, despite its therapeutic potential in this species. The objective of the present study was to determine the concentrations in the plasma and the pharmacokinetic and pharmacodynamic variables of enrofloxacin in goats after a single subcutaneous (s.c.) administration at a dose of 7.5 mg/kg body weight.

MATERIALS AND METHODS

Animals

The study was conducted in 5 adult female goats of nondescript breed, with an average weight of 20 kg, that were obtained from the Livestock Farm, Indian Veterinary Research Institute, Izatnagar. All the animals were clinically normal and they were housed in animal sheds with a concrete floor. They were given concentrates, green fodder and roughage and had free access to water.

Drug and treatment

Enrofloxacin (Enrocin, 10% injectable solution) was obtained from M/s Ranbaxy Laboratories, New Delhi, India. The drug was injected subcutaneously (7.5 mg/kg) in the right foreflank of each goat. The pure technical grade of enrofloxacin employed as the standard in the microbiological assay was a generous gift from M/s Intas Pharmaceuticals Pvt Ltd, Ahmedabad, India.

Sampling procedure

Blood samples (3–5 ml) were collected into heparinized tubes by jugular venepuncture, prior to and at 5, 10, 15, 30 and 45 min and 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24 and 48 h after drug administration. The plasma was separated after centrifugation at 950*g* for 20 min and stored at –20°C until assayed for enrofloxacin.

Microbiological assay

The concentrations of enrofloxacin in the plasma were determined by an agar well diffusion microbiological assay using *Escherichia coli* (ATCC 25922) as the test organism (Arret *et al.*, 1971). The diameters of the zones of bacterial inhibition were

measured and converted to concentrations of enrofloxacin equivalent by the use of a standard curve calibrated by adding known amounts of pure enrofloxacin to blank caprine plasma. Each test sample or standard was assayed in triplicate and the mean of three observations was determined. The limit of quantitation of the assay was 0.08 μg/ml.

Pharmacokinetic analysis

The plasma drug concentration–time data for each individual animal were analysed by non-compartmental techniques using the computer programme MicroPharm (Pharmacokinetics, Version 1.0, S. Urien, INSERM, Creteil, France, 1989). The programme calculated the area under the plasma concentration–time curve for the time at which the last drug concentration was measured (AUC_{0-t}) and the area under the first moment curve ($AUMC_{0-t}$), using the linear trapezoidal rule. The terminal rate constant (β) was obtained from the slope of a regression line calculated from the semilogarithmic plot of the last 6 or 7 concentrations of the plasma drug concentration–time curve. The AUC and AUMC were extrapolated to infinity from the last measured concentration and the terminal rate constant (Gibaldi and Perrier, 1982). The elimination half-life ($t_{\frac{1}{2}\beta}$) was given by

$$t_{\frac{1}{2}\beta} = (\ln 2)/\beta \tag{1}$$

The mean residence time (MRT) was calculated according to

$$\mathrm{MRT} = \mathrm{AUMC}/\mathrm{AUC} \tag{2}$$

The volume of distribution at steady-state (V_{dss}) was assessed from

$$V_{dss}/F = D \times \mathrm{AUMC}/(\mathrm{AUC})^2 \tag{3}$$

where D is the dose of the drug and F is the undetermined bioavailability. Similarly, the total body clearance (Cl_B) was assessed from

$$Cl_B/F = D/\mathrm{AUC} \tag{4}$$

The observed values were used to calculate the maximum plasma concentration of the drug (C_{max}) and the time to reach maximum plasma concentration (t_{max}).

RESULTS

The plasma concentration–time data for enrofloxacin after subcutaneous administration in goats are presented in Figure 1. An appreciable concentration of enrofloxacin (0.30 ± 0.13 μg/ml) had appeared in the plasma after 5 min. The pharmacokinetic parameters of enrofloxacin are presented in Table I.

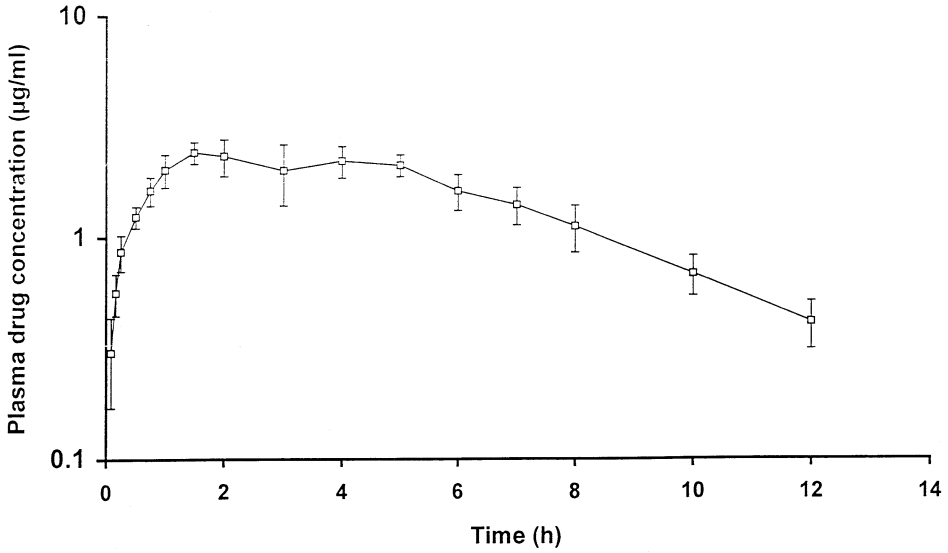

Figure 1. Semilogarithmic plot of mean (±SE) concentrations of enrofloxacin in plasma versus time following a single subcutaneous administration of enrofloxacin (7.5 mg/kg) in 5 goats

TABLE I
Pharmacokinetic parameters of enrofloxacin after single subcutaneous administration (7.5 mg/kg) in 5 goats

Kinetic parameter	Unit	Mean ± SE
$AUC_{0-\infty}$	µg.h/ml	19.35 ± 3.47
$AUMC_{0-\infty}$	µg.h^2/ml	114.54 ± 24.19
MRT	h	5.74 ± 0.28
β	h^{-1}	0.25 ± 0.03
$t_{\frac{1}{2}\beta}$	h	2.84 ± 0.57[a]
V_{dss}/F	L/kg	2.48 ± 0.37
Cl_B/F	L.h^{-1}/kg	0.45 ± 0.09
C_{max}	µg/ml	2.91 ± 0.39
t_{max}	h	2.9 ± 0.51

[a]Geometric mean ± SE

DISCUSSION

An attempt was made in the present study to characterize the pharmacokinetic behaviour of enrofloxacin in goats after single s.c. administration at a dose of 7.5 mg/kg. The same dose of enrofloxacin was used in previously reported pharmacokinetic studies in cattle (Stegemann *et al.*, 1997) and horses (Giguere *et al.*, 1996; Haines *et al.*, 2000). Enrofloxacin has been shown to partially biotransform to a pharmacologically active metabolite, ciprofloxacin, in several animal species (Kaartinen *et al.*, 1995, 1997a; Mengozzi *et al.*, 1996; Anadon *et al.*, 1999). The microbiological assay employed in the present study does not distinguish between enrofloxacin and its active metabolite ciprofloxacin, and so measured the total antimicrobial activity. The latter is adequate to determine dosage regimens and pharmacodynamic parameters correlated to the therapeutic efficacy of fluoroquinolones (Drusano *et al.*, 1993; Giguere *et al.*, 1996). The limit of quantitation of the assay procedure in the present study was similar to that reported recently by Haines and colleagues (2000) and the assay was 5.5 times more sensitive than that reported by Verma and colleagues (1999). Microbiological assays have also been used by other authors to measure the concentrations of enrofloxacin in the plasma/serum of animals (Gavrielli *et al.*, 1995; Kaartinen *et al.*, 1995, 1997a; Stegemann *et al.*, 1997; Verma *et al.*, 1999; Haines *et al.*, 2000).

Subcutaneous administration of enrofloxacin to goats in a single dose of 7.5 mg/kg resulted in a peak plasma drug concentration (C_{max}) of 2.91 ± 0.39 μg/ml at 2.9 ± 0.51 h. A lower C_{max} of 1.71 ± 0.93 μg/ml was reported in cattle given the same s.c. dose of enrofloxacin (Stegemann *et al.*, 1997). In a study by Kaartinen and colleagues (1995), the C_{max} was 0.98 ± 0.20 μg/ml at 3.2 ± 1.09 h in lactating cows following s.c. injection of enrofloxacin (5 mg/kg). A C_{max} of 1.23 ± 0.27 μg/ml at 1 h was reported in camels given enrofloxacin subcutaneously at one-third of the dose used in the present study (Gavrielli *et al.*, 1995). The appearance of a second peak in concentration of the drug in the plasma 4 h after administration of enrofloxacin may be a result of the enterohepatic recycling known to occur with some fluoroquinolones, including enrofloxacin in equids (Brown, 1996). The elimination half-life ($t_{\frac{1}{2}\beta}$) obtained for enrofloxacin in goats of 2.84 ± 0.57 h is much shorter than that reported for camels (10.58 ± 6.7 h) after s.c. administration of enrofloxacin at 2.5 mg/kg (Gavrielli *et al.*, 1995).

The AUC of enrofloxacin (19.35 ± 3.47 μg.h/ml) obtained in the present study is similar to the value reported in cattle (18.9 ± 2.8 μg.h/ml) after s.c. administration of enrofloxacin at 7.5 mg/kg (Stegemann *et al.*, 1997). The MRT determined in goats (5.74 ± 0.28 h) was markedly shorter than that reported in lactating cows (8.40 ± 0.87 h; Kaartinen *et al.*, 1995). The volume of distribution (V_{dss}/F) of 2.48 ± 0.37 L/kg indicated that enrofloxacin is widely distributed in tissues and body fluids of goats.

Fluoroquinolone antimicrobials exhibit a concentration-dependent bactericidal effect and it is therefore not necessary to maintain their plasma concentrations above the MIC throughout the entire dosing period. These drugs are likely to be highly effective when given in large doses at infrequent intervals (Boothe, 1994; Nightingale *et al.*, 2000). Taking into consideration the pharmacodynamic and pharmacokinetic properties of fluoroquinolones, it has been suggested that the critical breakpoints determining the therapeutic efficacy of these drugs are $C_{max}/MIC \geqslant 10$ and an area

under the inhibitory concentration curve AUIC (AUC/MIC) $\geqslant$100 (Nightingale *et al.*, 2000; Walker, 2000). Although the MIC values of enrofloxacin reported for the veterinary pathogens *Escherichia coli*, *Haemophilus somnus*, *Moraxella bovis* and *Salmonella* spp. are in the range 0.01–0.06 μg/ml, *Pasteurella multocida* and *Yersinia* spp. (usual MICs 0.008–0.01 μg/ml) are highly sensitive to this drug (Scheer, 1987; Prescott and Yielding, 1990; Bottner *et al.*, 1995). The usual therapeutic concentration (MIC) of enrofloxacin against most veterinary pathogens is reported to be equal to or below 0.1 μg/ml (Kaartinen *et al.*, 1997b). Using an MIC of 0.1 μg/ml, a single dose of 7.5 mg/kg of enrofloxacin yielded a C_{max}/MIC ratio of 29 and an AUIC of 198, indicating that this would be an effective dose.

Enrofloxacin concentrations above 0.1 μg/ml in the plasma persisted for more than 12 h in goats. Considering the pharmacokinetic and pharmacodynamic parameters determined in the present study, and the known post-antibiotic effect of fluoroquinolone antimicrobials, which lasts for 4–8 h (Boothe, 1994; Brown, 1996), a dose of 7.5 mg/kg of enrofloxacin given subcutaneously once daily can be recommended in goats. Additional studies will be needed to define the therapeutic efficacy and safety of this dosage under clinical circumstances.

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