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PHARMACOKINETIC BEHAVIOR OF PEFLOXACIN IN LACTATING GOATS

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SUMMARY

Pefloxacin (PEL) was administered intravenously (iv) and intramuscularly (im) at a dose rate of 10 mg kg^{-1} b.wt. to five healthy, lactating goats weighing 18-22 kg. Blood, urine and milk samples were collected at precise time intervals and the concentrations were determined by using a microbiological assay method. The pharmacokinetic parameters were estimated using routine equation. A two compartment open model best described the decrease of pefloxacin concentration in serum after an intravenous injection. The drug was rapidly and widely distributed with distribution half-life ($t^1/_2\alpha$ of 0.097 ± 0.012 h and steady-state volume of distribution (Vdss) 5.144 ± 0.206 L kg^{-1}. PEL was rapidly absorbed after im injection with an absorption half-life ($t^1/_2ab$) of 0.315 ± 0.016 h. The peak serum concentration Cmax was 0.862 ± 0.083 µg.ml^{-1} at Tmax 0.75 h. The systemic bioavailability after im injection was 70.632±1.130% and the serum protein bound fraction was 9.756 ± 1.573%. The drug was detected in milk and urine for 12 and 72 h, respectively.

INTRODUCTION

The first antimicrobials based on the 4-quinolone ring were nalidixic acid and oxolinic acids, which are active, *in vitro* against a wide range of Gram-negative bacteria. The included problems that associated with their application are restricted spectrum of activity and the relatively rapid emergence of resistant mutants. This led to the discovery of fluoroquinolones; one of them is pefloxacin. Pefloxacin is 1 - Ethyl - 6 - fluoro - 1,4 - dihydro - 7 - (4 - methyl - 1 - piperazinyl) - 4 - oxo - 3 - quinoline carboxylic acid. As with other fluoroquinolones, pefloxacin achieves rapid bactericidal activity by inhibiting the bacterial DNA gyrase (Chu and Fernandes, 1991). The drug possesses good *in vitro* activity against a variety of pathogens, including Gram-positive and

Gram-negative bacteria. Pefloxacin is relatively similar to other fluoroquinolones such as enrofloxacin, ciprofloxacin and marbofloxacin (Van-Custen et al., 1990; Spreng et al., 1995; Brown, 1996) in that they have a wide spectrum of activity, a large volume of distribution and are active at low concentrations. The pharmacokinetic properties of pefloxacin have not been reported in goats but evaluated in sheep (Moutafchieva and Djouvinov, 1997).

The great relevance of goats as food producing animals, and the lack of information regarding the pharmacokinetics of this antibacterial, have urged us to study its kinetic aspects in healthy goats following intravenous and intramuscular administration of a single dose as well as its excretion in milk and urine.

MATERIALS AND METHODS

Experimental animals:

Five clinically healthy lactating, Egyptian goats weighing 18-22 kg (2-year old) were used. Body weights were recorded for each animal on the day prior to initiation of the study. Animals were kept indoors under good hygienic condition, fed on alfalfa, hay, concentrated mixture in a pelted form and water ad-libitum. The study was conducted using a two-way crossover design with one-month interval between each experiment to ensure complete withdrawal of the drug from the body.

Experimental Design

Peflacine® (Rhone-Poulenc, Rorer, Paris, France) injectable solution was used. It was injected intravenously into the right jugular vein and intramuscularly into the lower third of the right cervical musculature at a dose rate of 10 mg kg^{-1} b.wt. Blood samples of about 3 ml each were collected from the contralateral vein just prior dosing and at 5, 10, 20, 30, 45 min and 1, 2, 4, 6, 8, 10, 12 and 24 h after drug administration by both routes. The blood was allowed to clot at room temperature for 2 h, then the serum was separated by centrifugation at (3000 *g*) for 15 min. Milk and urine samples were collected before and at 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 48 and 72 h post-injection following evacuation of both udder and bladder. All samples were stored at -20 °C until used for assessment.

Pefloxacin assay:

The concentrations of microbiologically active pefloxacin in serum, milk and urine were determined by an agar plate diffusion method (Bennett et al., 1966) using *E. coli (ATCC 8737)* as test organism, growing on Mueller-Hinton agar (Mast Group Ltd., Mersyside, UK). Six wells, 8 mm in diameter were cut at equal distances in standard Petri-dishes (120 x 120) containing 25 ml seeded agar. The wells were filled with 0.1 ml of either the test samples or pefloxacin standards. The plates were kept at room temperature for 2 h before incubation at 37°C for 16 -18 h. The diameters of the inhibition zone were measured and

concentrations in the test samples were calculated from the standard curve. Semilogarithmic plots of the diameter of the inhibition zone-vs-standard pefloxacin the concentrations in serum ranging from 0.078 $\mu g.ml^{-1}$ to 2.5 $\mu g.ml^{-1}$ were linear with typical correlation coefficient of 0.989 (for the standard curve). The limit of pefloxacin quantitation in serum was 0.078 $\mu g.ml^{-1}$

The extent of pefloxacin binding to serum proteins of goats was determined *in vitro* by the method previously reported by (Craig and Suh 1980) using antimicrobial-free goat's serum fortified with known concentration of pefloxacin, 1.25, 0.625, 0.312 and 0.156 $\mu g.ml^{-1}$.

Pharmacokinetic analysis

A computerized curve-stripping program (R Strip, Micromath Scientific Software, Salt Lake City, UT, USA) was used to analyze the concentration-vs-time for each individual goat after the administration of pefloxacin by both routes. Following intravenous injection, the disposition curves of pefloxacin that express the decline in drug concentration as a function of time was best described by a two -compartment open model:

$Cp^{\circ} = Ae^{-\alpha t} + Be^{-\beta t}$

Cp°= The concentration of drug in plasma at time t.

A= Intercept of the distribution phase with the concentration axis expressed as $\mu g.ml^{-1}$.

B= Intercept of the elimination phase with the concentration axis expressed as $\mu g.ml^{-1}$.

α = Distribution rate constant expressed in units of reciprocal time (h^{-1}).

β = Elimination rate constant expressed in units of reciprocal time (h^{-1}).

e = Base of natural logarithm.

RESULTS

It should be noticed that pefloxacin administration to lactating goats did not produce any adverse clinical signs or side effects. The obtained data showed that, following a single intravenous administration, pefloxacin concentration decreased in a biexponential manner and follows a two-compartment open model (Figure 1). Pharmacokinetic values are summarised in Table 1. The half-lives of distribution ($t_{1/2}(\alpha)$) and elimination ($t_{1/2}$) were 0.097 ± 0.012 a nd 1.595 ± X 0.301 h, respectively. The steady state volume of distribution (Vdss) was 5.144 ± 0.206 L. kg^{-1} and mean residence time (MRT) was 1.072 ± 0.129 h. Following the intramuscular injection, the peak serum level (0.862 ± 0.083 $\mu g.ml^{-1}$) was achieved at 0.75 h post-injection (Figure 2). The drug was detected in a therapeutic concentration for 8 h in serum and 10 h in milk. However, pefloxacin was detected in urine for 48 h post- injection. The systemic bioavailability of pefloxacin following im administration was 70.632 (1.130% whereas; the extent of protein binding was 9.756 ± 1.573 %.

Table (1): Mean (SEM) kinetic parameters of pefloxacin following a single intravenous and intramuscular injection of 10 mg kg^{-1} b.wt. in goats,(n=5)

Parameter	Unit	Intravenous	Intramuscular
$t^1/_{2\alpha}$	h	0.097-0.012	0.315±0.016
$t^1/_{2ab}$	h	NA	2.199±0.142
k_{ab}	h^{-1}	NA	1.512±0.186
$t^1/_{2\beta}$	h	1.595±0.301	NA
k_{12}	h^{-1}	3.309±0.465	NA
k_{21}	h^{-1}	0.939±0.353	
k_{12}/k_{21}	ratio	3.523	NA
Vd_{ss}	$L.kg^{-1}$	5.144±0.206	NA
Vd(area)	$L.kg^{-1}$	8.282±1.128	NA
Cl_{tot}	$L.kg^{-}.h^{-1}$	3.594±0.303	0.862±±0.083
Tmax	$\mu g.ml^{-1}$	NA	0.75
AUC	h	NA	1.965±0.127
AUMC	$\mu g.ml.h^{-1}$	2.782±0.224	3.776±0.543
MRT	$\mu g.ml.h^{-2}$	2.628±0.395	2.140±0.192
F	h	1.072±0.129	70.632±1.13
	%	NA	

NA, not applicable, S.E., standard error

$t^1/_{2\alpha}$: distribution half-life, $t^1/_{2\beta}$: elimination half-life, K_{12} and K_{21} first-order rate constants for drug distribution between the central and peripheral compartments, $Vd_{(ss)}$: volume of distribution at steady state, Cl_{tot} total body clearance, C_{max} peak drug concentration, T_{max} time to peak concentration, K_{ab} absorption rate constant, $t^1/_{2ab}$ absorption half-life,$T_{1/2ab}$ elimination half-life, AUC area under the curve from zero to infinity by the trapezoidal integral, AUMC area under the first moment curve and MRT mean residence time in plasma . F the systemic bioavailability calculated as $(AUC_{i.m.}/AUC_{i.v.})X\ 100$.

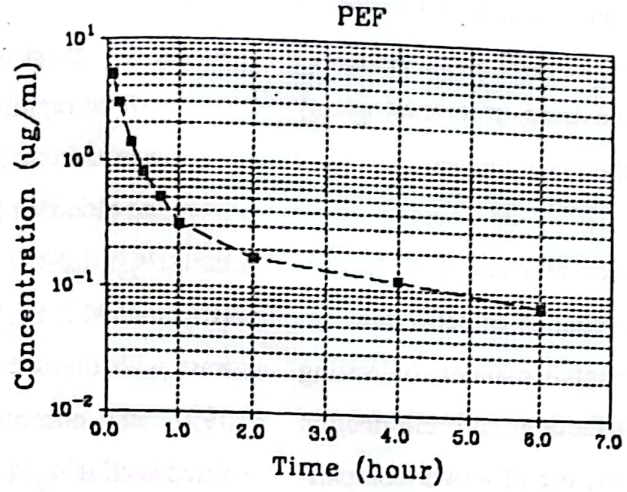

Fig. (1): Semilogarithmic graph depicting the time serum concentration curve of perfloxacin following intravenous injection of 10 mg kg^{-1} b.wt. in lactating goats (n=5).

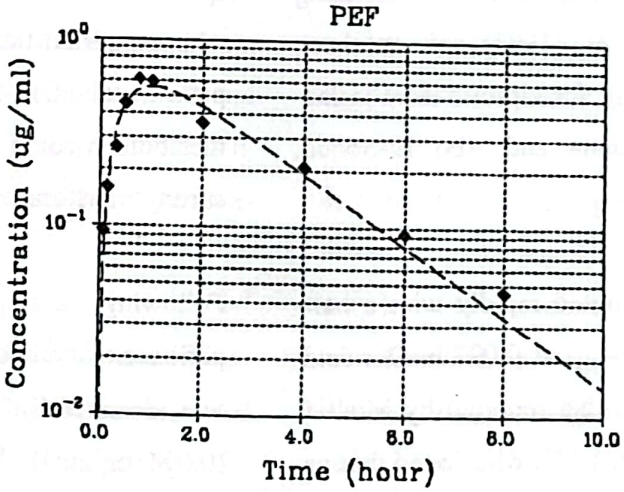

Fig. (2): Semilogarithmic graph depicting the time serum concentration curve of perfloxacin following intramuscular injection of 10 mg kg^{-1} b.wt. in lactating goat s (n=5).

DISCUSSION

Interpretation of the data gathered in the course of the present study must be taken into consideration the assay method used (microbiological) and the sensitivity of the assay method.

The present investigation revealed that, the serum concentration time courses of pefloxacin decreased in a bi-exponential manner, indicating the presence of distribution and elimination phases and justifying the use of a two- compartment kinetic model for analyzing the data following intravenous injection of 10 mg kg ñ1 b.wt. Our finding was closely observed in sheep (Moutafchieva and Djouvinov, 1997), for danofloxacin in cattle (Giles et al., 1991a), in lactating ewes (Shem-Tov et al., 1997a), in lactating cows (Shem-Tov et al., 1998) and in sheep (Mckellar et al., 1998), for ciprofloxacin in lactating goats (El- Banna and Abo El-Sooud, 1998).

Pefloxacin was distributed rapidly with a half-life of distribution ($t^1/_{2ab}$) of 0.097 h. Our finding was variable with that reported by Moutafchieva and Djouvinov, 1997, who found that pefloxacin was slowly distributed. Whereas, these finding were invariable with that recorded for danofloxacin both in lactating ewes, 0.208 h (Shem-Tov et al., 1997a) and lactating cows, 11.42 min (Shem -Tov et al., 1998) for ciprofloxacin in lactating goats 13.5 min. (El- Banna and Abo El-Sooud, 1998) and marbofloxacin in lactating cows and ewes, 11.88 and 14.16 mmin., respectively (Shem -Tov et al., 1997b).

As the drug persisted in the central compartment, so it will be rapidly metabolized and eliminated. This was evidenced by its rapid disappearance from the blood with a short half - life of elimination ($t^1/_{2\beta}$) 1.595 h and high total body clearance (Cl_{tot}) 3.594 L kg. h^{-1}. These results were in contrast with that of Moutafchieva and Djouvinov 1997, who observed that pefloxacin was eliminated with ($t^1/_{2\beta}$) (of 6.88 h in sheep but consistent with that of danofloxacin in lactating ewes, 2.08 h (Shem - Tov et al., 1997a), marbofloxacin in lactating cows and ewes, 2.06 and 2.18 h (Shem - Tov et al., 1997b). The relatively shorter half-life of elimination in goats than any other species is discussed by Hennessy et al. (1993), who suggested that goats possesses a faster hepatic metabolism than sheep. The faster hepatic metabolism could be attributed to its higher glucuronyltransferase (Short et al., 1988).

Following a single intramascular injection of pefloxacin at a dose of 10 mg kg^{-1} b.wt, the drug was detected in serum 5-min after injection (0.094 µg.ml^{-1}). The peak serum value 0.862 µg.ml^{-1}) was achieved at 0.75 h after injection. The serum concentration decreased gradually till reaching the lowest detectable level 0.084µgm-1-8h post-injection. These findings were consistent with that reported by (Pugliese et al ., 1991), who found that enrofloxacin was detected in serum for up to 4 h after intramuscular

administration in sheep. In this respect, the serum pefloxacin level in goats suggested its therapeutic effectiveness till the 8th h following injection.

Pefloxacin was rapidly absorbed from the intramuscular site of injection with absorption half-life ($t^1/_{2ab}$) 0.315 h and absorption rate constant (Kab 2.199 h^{-1} evidenced by shorter Tmax (0.75 h). The peak serum concentration (C_{max}) was 0.862 $\mu g.ml^{-1}$. The peak serum concentration were consistent with that recorded for danofloxacin both in cattle (Giles et al., 1991) and sheep (Mckellar et al., 1998), and differ from that of pefloxacin in sheep, 3.58 $\mu g.ml^{-1}$(Moutafchieva and Djouvinov, 1997) and ciprofloxacin in lactating goats, 1.231 ug ml^{-1} (El-Banna and Abo El-Sooud, 1998). On the other hand, Shem-Tov et al., 1997a and 1998 found that the maximum concentrations of danofloxacin (Cmax) 0.80 and 1.37 $\mu g.ml^{-1}$ were attain at (Tmax) 4 and 5.78 h, in lactating ewes and lactating cows, respectively.

It has been noticed that the half-life of elimination ($t^1/_{2\beta}$) 1.512 h was shorter than that reported in sheep (Moutafchieva and Djouvinov, 1997). This was explained by Baggot (1992) who noted that the half - life values of some drugs eliminated by hepatic metabolism are shorter (by about 2 folds) in goats than others. He found that pygmy (dwarf-like) goats metabolize phenazone (microsomal oxidation), sulphonamides (hydroxylation) and chloramphenicol (glucuronide synthesis) more than other breeds.

Urine concentration of pefloxacin after both intravenous and intramuscular injection exceeded the MIC of most sensitive urinary tract pathogens. Our finding suggest that pefloxacin would be effective in the eradication of many urinary tract pathogens.

The systemic bioavailability of pefloxacin in goats after intramuscular injection of 10 mg kg^{-1} b.wt, was 70.632 %. This finding indicate a good absorption of pefloxacin from the site of injection. This finding agree with that reported for pefloxacin in sheep 82.42% (Moutafchieva and Djouvinov, 1997) and in contrast to that recorded for danofloxacin by Shem -Tov et al., 1997a and 1998 in lactating ewes and lactating cows >100%. This may be due to species variations, age and regional blood flow.

In vitro protein binding percent of pefloxacin in serum of goats was 9.756 %, indicated that the drug has a low tendency to bind the plasma protein. This finding was reported by (El Bahri and Blouin, 1991), who found that, fluoroquinolones have a low tendency (15% to 20%) to bind to plasma protein. This finding was consistent with that of ciprofloxacin in goats, 14.2 % (El-Banna and Abo El-Souud, 1998). It was stated that fluoroquinolone binding tendency to serum protein is relatively low up to 30% (Wise et al., 1984). This variation may be due to species differences, and the assay method used (Haddad et al., 1985).

ULTIMATELY

Since the drug was recovered in serum for only a short period, it may not be of much use in cases of septicemia as multiple doses or high loading and maintenance doses would be required to maintain this concentration in serum. Furthermore, since it is eliminated at very high concentrations in urine, this drug would be extremely useful for treating suitable urinary tract infections.

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