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RENAL CLEARANCE AND URINARY EXCRETION OF CIPROFLOXACIN IN GOATS

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ABSTRACT

The renal clearance and urinary excretion of ciprofloxacin were investigated in eight healthy female goats. In each animal, ciprofloxacin was administered intramuscularly at the rate of 5 mg/kg body weight. Following drug administration, blood and urine samples were collected at different time intervals and analyzed for ciprofloxacin and creatinine. High performance liquid chromatography (HPLC) was used to determine the drug concentration in the plasma and urine. The value of diuresis after single administration of ciprofloxacin was 0.073 ± 0.014 ml/min/kg. Mean (± SE) values for renal clearance of creatinine and ciprofloxacin were 1.870 ± 0.385 and 0.982 ± 0.166 ml/min/kg, respectively. The ratio between the renal clearance of ciprofloxacin and that of creatinine remained less than one, which was indicative of back diffusion. The mean (± SE) value for the cumulative percent of ciprofloxacin dose excreted at 10 hours following its intramuscular administration was 13.03 ± 2.07. Based on these results, it was evident that besides glomerular filtration, renal handling of drug involved back diffusion also. It was concluded that in local goats glomerular filtration rate (GFR) was lower than that reported for their foreign counterparts.

Key words: Renal clearance, urinary excretion, ciprofloxacin, goats.

INTRODUCTION

Several studies have shown that the pharmacokinetic behavior, optimal dosage, renal clearance and urinary excretion of various drugs are different under indigenous conditions when compared with the values given in the literature or in the product inserts supplied by the manufacturers (Nawaz *et al.*, 1988; Muhammad, 1997; Javed *et al.* 2003; Javed *et al.* 2005 a and b).

The fluoroquinolones are a series of synthetic antibacterial agents which are used for the treatment of a variety of bacterial infections. All the fluoroqui-nolones exhibit such distributional and antimicrobial properties that make them potentially useful in veterinary medicine. They have extensive application in clinical practices because of their good bioavailability and pharmacokinetic profile, arousing great interest in the field of chemotherapy (Vancutsem *et al.*, 1990). Ciprofloxacin is an important member of fluoroquinolone group of antibiotics. It is a broad spectrum antibiotic, being used to combat various infectious diseases in man and animals (Stein, 1996). However, biodisposition of ciprofloxacin has not been studied in local ruminant species. Keeping in view the vast clinical use of ciprofloxacin in local animals, the present study was designed to determine the renal clearance and urinary excretion of this drug in goats.

MATERIALS AND METHODS

Experimental animals and treatments

Renal clearance and urinary excretion of ciprofloxacin was investigated in eight healthy adult female goats during the month of December, 2006. The average body weight of the goats was 35.38 ± 1.97 kg. All the goats were maintained under similar environmental and managemental conditions at the Experimental Farm, Department of Livestock Management, University of Agriculture, Faisalabad, Pakistan. The animals were fed with seasonal green fodder and had free access to drinking water.

In all animals, control blood and urine samples were collected before the drug administration. A commercial injectable preparation of ciprofloxacin (CIPROCIN-100®, Han Dong Corporation Ltd., Korea) was given intramuscularly at the rate of 5 mg/kg body weight to each animal. Following drug administration, the blood samples were collected at 1.0, 1.5, 2.0 and 2.5 hours in plastic centrifuge tubes. The pH of fresh blood samples was recorded using an electronic pH meter (Beckman HS, Germany) with a glass electrode at 37^0C. Blood samples were centrifuged, plasma was separated and stored at -20^0C until analysis.

Renal clearance

The left jugular vein of each goat was cannulated with a plastic canula (No. 90, Protex Ltd., England). Sterilized disposable balloon catheter (Rush No. 14, 30

ml) was inserted into urinary bladder through urethra of each animal after lubrication with paraffin gel. The external opening of catheter was connected through rubber tubing to a reservoir in which all the voided urine was quantitatively collected.

At 45 minutes following drug administration, the urinary bladder was emptied completely and washed with distilled water through the catheter. After washing, urine samples were collected at 75, 105, 135 and 165 minutes after drug administration. The volume of each urine sample was measured. Ciprofloxacin concentrations in plasma and urine samples collected at different time intervals post medication were determined by using HPLC. The creatinine concentrations in plasma and urine samples were determined by using reagent kit (Thomas, 1998), with the help of BTS-330 (Bio-Systems S.A., Spain) according to Jaffe reaction. Renal clearance of ciprofloxacin and endogenous creatinine was calculated. The renal clearance of endogenous creatinine was used for the estimation of glomerular filtration rate (GFR).

Urinary excretion

For the determination of urinary excretion of ciprofloxacin, the urine samples were collected for the drug assay before and at 4, 6, 8 and 10 hours interval after drug administration. The pH of all urine samples was recorded. Cumulative percent of the dose of ciprofloxacin in the urine until 10 hours following its intramuscular administration was calculated.

HPLC analysis

Chromatography was performed with a High Performance Liquid Chromatograph (Sykam, S-1122) and analytes were determined using UV/Vis detector (Sykam, S-3210). The output of the detector was monitored with computer software (Peak Simple Chromatography Data System, Buck Scientific Inc., East Norwalk). A stainless steel column packed with YMC pack A-312 (Thermo Hypersil-Keystone, BDS-C_{18} with 250 x 4.6 mm dimensions and 5µm particle size) was used. The column was protected with a pre-column (Guard-Pak™) filled with a µBondapak™ C_{18} cartridge (Thermo Hypersil, England). Separation of ciprofloxacin was achieved at 37^0C, using an isocratic mode. The mobile phase consisted of a mixture of 800 ml of 14 ml/L phosphoric acid and 200 ml of acetonitrile per liter. The UV detector was set at 275 nm and the flow rate was 1 ml/min.

For preparation of plasma samples, 2 ml of acetonitrile was added to 1 ml of plasma, plasma blank or plasma calibrator in a centrifuge tube. The mixture was vortexed for one minute and centrifuged for 30 minutes at 4000 rpm. The supernatant was transferred to a glass tube and the liquid phase was evaporated to dryness in a boiling water bath. The residue was then reconstituted in 10 µl of internal standard and 1 ml of 14 ml/L phosphoric acid. The final solution was again vortexed for 30 seconds, filtered and 20 µl was injected into the HPLC system. However, the urine samples were diluted 1:20 (by volume) with the mobile phase. In a centrifuge tube, 10 µl of the working solution of paracetamol was added to 1 ml of the diluted urine. The mixture was vortexed, filtered and 20 µl was injected directly into the HPLC system. This method, previously described by Soback *et al.* (1994), was partially modified and validated. The ciprofloxacin recovery was 76% and coefficient of variation was <2% for intra and inter assays differences. The limit of detection was 75 ng/ml, while the limit of quantitation was 250 ng/ml.

Statistical analysis

The mean (± SE) values for concentration of each metabolite were calculated. For the assessment of renal handling of ciprofloxacin following its intramuscular administration, influence of urine pH, rate of urine flow (diuresis) and plasma drug concentration on its renal clearance was examined by regression/correlation analysis using Microsoft Excel version, 2003.

RESULTS AND DISCUSSION

Renal clearance

The results of diuresis, blood and urine pH, renal clearance of creatinine and ciprofloxacin are presented in Table 1. The rate of urine flow (diuresis) in 8 goats following ciprofloxacin administration was 0.073 ± 0.014 ml/min/kg. In previous studies on Danish goats, the value of diuresis was 0.052 ml/min/kg (Rasmussen *et al.*, 1975), 0.049 ml/min/kg (Nawaz and Rasmussen, 1979), in Pakistani goats 0.026 ml/min/kg (Shah *et al.*, 1983), 0.084 ml/min/kg (Javed *et al.*, 1984), 0.130 ml/min/kg (Iqbal *et al.*, 1986), 0.052 ml/min/kg (Nawaz *et al.*, 1990) and 0.121 ± 0.015 ml/min/kg (Iqbal, 1994). The rate of urine flow depends upon several factors like water intake, metabolic status of animal and environmental conditions.

The mean (± SE) value for the pH of blood was 7.29 ± 0.02, while that of the urine it was 8.18 ± 0.01. The value of blood pH is within the range of values recorded in domestic ruminants including goats (Iqbal *et al.*, 1986; Nawaz *et al.*, 1988; Nawaz *et al.*, 1990; Javed *et al.*, 2005b). The distribution of a drug across biological membranes of various body compartments is determined by the physicochemical characteristics of the drug and pH of environment across the biomembrane (Baggot, 1977). The unionized moiety of

Table 1: Mean (± SE) values for body weight, diuresis, plasma and urine concentration and renal clearance of endogenous creatinine and ciprofloxacin in 8 goats following intramuscular administration of ciprofloxacin at dose rate of 5 mg/kg body weight

Animal No.	Body weight (kg)	Diuresis (ml/min/kg)	pH		Creatinine conc. (µg/ml)		Ciprofloxacin conc. (µg/ml)		Renal clearance (ml/min/kg)		Ratio Cip/Creat
			Blood	Urine	Plasma	Urine	Plasma	Urine	Creat.	Cipro.	
1	42	0.123	7.37	8.14	12.00	312.5	1.37	8.16	3.016	0.832	0.28
2	35	0.058	7.28	8.19	15.50	425.0	1.14	11.36	1.490	0.609	0.41
3	27	0.046	7.20	8.22	14.25	337.5	1.12	13.43	1.121	0.525	0.47
4	35	0.061	7.33	8.15	15.75	350.0	1.02	14.10	1.410	0.893	0.63
5	31	0.140	7.27	8.19	14.50	362.5	1.84	21.72	3.851	1.628	0.42
6	42	0.064	7.20	8.22	14.75	387.5	1.06	22.43	1.679	1.592	0.95
7	38	0.041	7.37	8.18	15.75	350.0	2.22	25.04	0.898	0.468	0.52
8	33	0.050	7.29	8.19	13.25	387.5	1.24	32.60	1.497	1.305	0.87
Mean	35.38	0.073	7.29	8.18	14.47	364.1	1.38	18.60	1.870	0.982	0.57
± SE	1.97	0.014	0.02	0.01	0.50	13.2	0.15	2.90	0.385	0.166	0.08

the drug can diffuse through biomembrane with ease and at equilibrium its concentration is alike on either side of the membrane. For acidic drugs, ionized moiety should be higher in the basic media or side of higher pH value. Thus, with decreasing alkaline pH, ionized moiety of the acidic drug would decrease, showing partly attribution to suitability for absorption.

The goats used in the present study showed a mean value of 1.87 ± 0.385 ml/min/kg for the renal clearance of endogenous creatinine. This value was lower than 2.62 ml/min/kg in Danish goats (Atef and Rasmussen, 1975), 2.5 ml/min/kg (Rasmussen *et al*., 1975) and 2.15 ml/min/kg (Nawaz and Rasmussen, 1979). However, this value was comparable to the values recorded in local goats i.e. 1.75 ml/min/kg (Shah *et al.*, 1983), 1.11 ml/min/kg (Javed *et al.*, 1984), 1.43 and 1.86 ml/min/kg during summer and winter, respectively (Nawaz *et al.*, 1988), 1.62 ml/min/kg (Nawaz *et al.*, 1990), 1.22 ml/min/kg (Raoof, 1989) and 1.29 ml/min/kg (Iqbal, 1994). These results indicate that GFR in local goats is lower than that in their foreign counterparts.

Mean (± SE) values for renal clearance of creatinine and ciprofloxacin were 1.870 ± 0.385 and 0.982 ± 0.166 ml/min/kg, respectively. The ratio between the renal clearance of ciprofloxacin and that of creatinine remained less than one, which was indicative of back diffusion.

The analysis of data on diuresis, urine pH, plasma concentration of ciprofloxacin and renal clearance of endogenous creatinine and ciprofloxacin showed a significant ($P<0.05$) positive correlation ($r = 0.420$) between diuresis and renal clearance of the drug which is indicative of the back diffusion (Fig 1). However, plasma concentration of ciprofloxacin and urine pH did not influence the renal excretion of the drug. Hence, renal handling of ciprofloxacin in goats involved glomerular filtration and back diffusion. However, in rabbits, dogs and humans, although active tubular secretion was involved, yet the renal clearance of quinolonecarboxylic acid mostly took place through glomerular filtration (Shimada *et al.*, 1983).

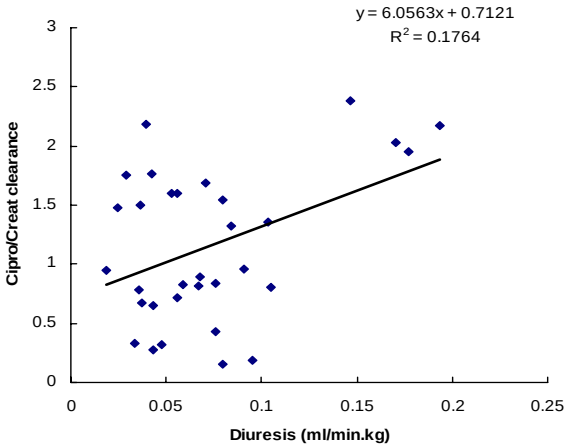

Fig. 1: Effect of diuresis of ciprofloxacin on its renal clearance in goats. Each data point shows one of the 32 observations in 8 experiments, each comprised of 4 experimental periods.

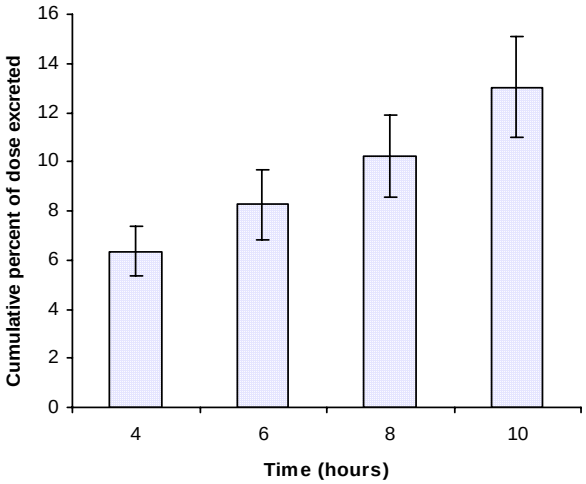

Fig. 2: Mean (± SE) values for cumulative percentage of ciprofloxacin intramuscular dose (5 mg/kg) excreted in urine of 8 goats at different time intervals.

Urinary excretion

Mean (± SE) values of urinary excretion in terms of cumulative percent of ciprofloxacin dose excreted in the urine at 4, 6, 8 and 10 hours following intramuscular administration have been presented in Fig 2. At 10 hours post drug administration, the value for the cumulative percent of ciprofloxacin dose excreted in the urine was 13.03 ± 2.07.

About 40% of the intravenous dose of norfloxacin was reported to be recovered in the urine of donkeys (Lavy *et al.*, 1995). As reported by Montay *et al.* (1984), the urinary recovery of pefloxacin was 29% of its dose in mice, 37.8% in rats, 36.3% in dogs, 26.5% in monkeys and 58.9% in humans. Lower urinary excretion of ciprofloxacin in goats may be related to its renal handling. In goats regardless of the glomerular filtration, the administered dose of ciprofloxacin was also absorbed at kidney tubular level through back diffusion. Moreover, lower GFR in goats may be responsible for the lower urinary excretion of this drug. (Hasan, 1998). Since ciprofloxacin is zwitterions at

physiological pH, the pKa values have been reported as 6.1 and 7.8 at its carboxylic and amino groups, respectively. It is at isoelectric point that ciprofloxacin is most lipophillic (Takacs-Novak *et al.,* 1992). Considering ciprofloxacin as weakly basic drug with pKa value of 7.8, 50% of the drug would be unionized at pH 7.8. The unionization of the drug would increase with increase in the pH of urine in domestic ruminants. A high degree of unionization provides more drug available for reabsorption and therefore, high clearance values may not be observed when pH of urine is high and this correlates with the lower urinary excretion in the present study.

Conclusions

Based on the findings of the present study, it was concluded that renal handling of ciprofloxacin, besides glomerular filtration, involved back diffusion also. Moreover, lower urinary excretion of ciprofloxacin was attributed to lower GFR and back diffusion at the kidney tubular level.

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