RENAL CLEARANCE AND URINARY EXCRETION OF NORFLOXACIN IN SHEEP

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ABSTRACT

The renal clearance and urinary excretion of norfloxacin was investigated in eight healthy sheep. In each animal, norfloxacin 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 norfloxacin and creatinine concentrations. Microbiological assay and spectrophotometer were used to measure drug and creatinine concentrations in plasma and urine samples, respectively. The value of diuresis after single administration of norfloxacin was 0.023 ± 0.004 ml/min.kg. Mean (± SE) values for renal clearance of creatinine and norfloxacin were 0.042 ± 0.005 and 0.013 ± 0.002 ml/min.kg, respectively. Keeping in view the results, it is clear that renal handling of norfloxacin involved active tubular secretion. The ratio between the renal clearance of norfloxacin and that of creatinine remained less than one, which is indicative of back diffusion. The mean (± SE) value for the cumulative percent of dose of norfloxacin excreted at 12 hours following administration of norfloxacin was 1.86 ± 0.05. Based on these results, it is evident that besides glomerular filtration, renal handling of drug also involved back diffusion and active tubular secretion. It was concluded that in local sheep glomerular filtration rate (GFR) was found lower than that of their foreign counter parts, reflected through less urinary excretion of norfloxacin.

Key words: Norfloxacin, renal clearance, urinary excretion, sheep.

INTRODUCTION

Most of the developing countries like Pakistan are importing raw and finished drugs for their human and animal health care programmes. Extensive pre-clinical or clinical investigations are carried out in drug exporting countries. Several studies have shown that pharmacokinetic parameters and optimal dosage regimens of investigated drugs were different under different indigenous conditions when compared with the values given in the literature or product inserts supplied by the manufacturers (Nawaz, 1994; Javed *et al.*, 2003; Muhammad *et al.*, 2003). In most cases the genetic make up of indigenous species and environmental conditions are different from their foreign counterparts and thus affect the biodisposition of drugs. Therefore, pharmacokinetic studies are important for suggesting optimal therapeutic regimen of a drug (Nawaz *et al.*, 1988)

Antibiotics play a vital role in the treatment of various infectious diseases. Third generation quinolones have extensive application in clinical practices because of their good bioavailability and pharmacokinetic profile and thus arousing great interest in the field of chemotherapy. Keeping in view the vast clinical use of norfloxacin in local animals, the present study was designed to determine the renal clearance and urinary excretion of this drug in sheep. It is hoped that the study will provide an insight into the mechanism involved in the renal handling of norfloxacin and its urinary excretion in one of the local domestic ruminant species.

MATERIALS AND METHODS

Experimental animals

Renal clearance and urinary excretion of norfloxacin was investigated in eight healthy adult female sheep. The average weight of the sheep was 42 Kg (34-48 Kg). All the sheep 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 green fodder of the season and had free access to drinking water. Experiments were conducted during the months of June and July 2004.

Renal clearance

In each animal left jugular vein was cannulated under strict aseptic conditions with 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 jell. The external opening of catheter was connected through rubber tubing to a reservoir in which all the voided urine was quantitatively collected. In all animals, control blood and urine samples were collected before the drug administration. A commercial preparation of norfloxacin (Norflox-50®, Kakasian Pharmaceuticals, Rawalpindi, Pakistan) was used. Norfloxacin was administered intramuscularly at the rate of 5 mg/kg body weight. Following drug administration, the blood samples were collected at 1.0, 1.5, 2.0 and 2.5 hours in heparinized 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°C. Blood samples were centrifuged; plasma was separated and stored at -4°C until analysis.

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. The volume of each urine sample was measured. Norfloxacin concentrations in plasma and urine samples collected at different time intervals post medication were determined following a "Microbiological Assay" (Arret *et al.*, 1971) according to disk agar diffusion method using sensitive microorganism *E. coli* as test organism. The creatinine concentrations in plasma and urine samples were determined according to the method of Bonsnes and Taussky (1945) by Jaffe reaction. Renal clearance of norfloxacin and endogenous creatinine was calculated. The renal clearance of endogenous creatinine was used for the estimation of glomerular filtration rate (GFR). For the assessment of the renal handling of norfloxacin following its administration, influence of urine pH, rate of urine flow (diuresis) and plasma drug concentration on the renal clearance of drug was examined by regression/correlation analysis.

Urinary excretion

For the determination of the urinary excretion of norfloxacin, the urine samples were collected for the drug assay before and at 6, 8 and 12 hours interval after drug administration. The concentration of norfloxacin in urine was determined by "Microbiological Assay" (Arret *et al.*, 1971) using sensitive microorganism *E. coli* as test organism. The pH of all urine samples was recorded. The mean (± SE) values for the norfloxacin in the urine samples at different time intervals were calculated. Cumulative percent of the dose of norfloxacin excreted in the urine until 12 hours following its intramuscular administration was calculated.

Statistical analysis

The mean (± SE) values for each concentration were calculated. The relationship between the urine flow, blood pH and plasma concentration of the drug was calculated with regression correlation analysis using Microsoft Excel version, 97.

RESULTS AND DISCUSSION

Renal clearance

Renal clearance of endogenous creatinine and norfloxacin was investigated following a single intramuscular administration of norfloxacin. The renal clearance of endogenous creatinine was measured as an index of glomerular filtration rate (GFR). The respective results of diuresis, blood and urine pH, renal clearance of creatinine and norfloxacin are presented in Table 1. The rate of urine flow (diuresis) in 8 sheep following single administration of norfloxacin was 0.023 ± 0.004 ml/min.kg. It was lower than earlier recorded values of 0.034 ml/min.kg (Afzal *et al.*, 1981), 0.054 ml/min.kg (Aslam, 1993) and 0.065 ± 0.01 ml/min.kg (Iqbal *et al.*, 1986). The rate of urine flow depends upon several factors like water intake, metabolic status of animal and environmental conditions.

Table 1: Renal clearance of endogenous creatinine and norfloxacin in sheep following single norfloxacin intramuscular administration (5 mg/kg body weight)

Animal No.	Body weight (kg)	Diuresis (ml/min.kg)	pH		Concentration (µg/ml)				Renal clearance (ml/min.kg)		Ratio cl Nor./ cl Creat.
			Blood	Urine	Creatinine		Norfloxacin		Creat.	Norfloxacin	
					Plasma	Urine	Plasma	Urine			
1	35	0.016	7.48	8.77	3.69	6.05	3.99	2.40	0.028	0.009	0.321
2	34	0.019	7.59	8.71	2.69	5.31	4.47	2.57	0.044	0.012	0.027
3	42	0.025	7.51	8.87	3.15	5.87	4.55	2.51	0.064	0.014	0.022
4	43	0.015	7.55	8.74	3.85	5.00	3.91	2.73	0.023	0.010	0.435
5	48	0.049	7.52	8.63	3.52	6.10	3.85	2.58	0.050	0.027	0.500
6	45	0.012	7.53	8.79	3.86	6.12	4.05	2.51	0.023	0.006	0.261
7	47	0.024	7.48	8.82	3.38	6.29	4.07	2.53	0.050	0.012	0.240
8	43	0.028	7.45	8.76	3.87	5.77	4.43	2.34	0.055	0.012	0.218
Mean	42	0.023	7.51	8.76	3.50	5.81	4.17	2.52	0.042	0.013	0.308
± SE	1.82	0.004	0.02	0.03	0.15	0.16	0.10	0.04	0.005	0.002	0.050

Each data point is mean of four observations in four experimental periods.

The pH of blood following single administration of norfloxacin was 7.51 ± 0.02. The values of blood pH recorded earlier in sheep during summer and winter were 7.60 and 7.74, respectively (Nawaz and Shah, 1984). The pH of urine following administration of norfloxacin was 8.76 ± 0.03. The pH of urine in sheep has been reportred as 8.45 and 8.47 during summer and winter seasons, respectively (Akhtar, 1987).

The distribution of a drug across biological membranes of various body compartments would be determined by the physiochemical characteristics of the drug and pH of environment across the biomembrane. Norfloxacin is a weakly acidic drug. The unionized moiety of the drug can diffuse through biomembrane with ease and at equilibrium its concentration is alike on either side of the membrane. For the 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 mean (± SE) value for concentration of endogenous creatinine in plasma following administration of norfloxacin was 3.50 ± 0.15 µg/ml. This value was lower than 15.24 ± 0.779 µg/ml reported earlier for sheep (Aslam, 1993). Plasma creatinine concentration recorded in the present study is lower than 12.3 µg/ml reported by Alvi *et al.* (1985) and 12.5 µg/ml by Akhtar (1987).

The mean (± SE) value for the renal clearance of creatinine in eight sheep following a single intramuscular administration of norfloxacin was 0.042 ± 0.005 ml/min.kg. This value is lower than previously reported values in sheep, 1.11 ml/min.kg (Afzal *et al.*, 1981), 1.18 ml/min.kg (Alvi *et al.*, 1985), 1.13 ml/min.kg (Akhtar, 1987) and 1.19 ml/min.kg (Ahmad, 1983).

Mean (± SE) value for the renal clearance of norfloxacin following its single administration in eight sheep was 0.013 ± 0.002 ml/min.kg. This value is lower than 0.029 ± 0.01 ml/min.kg observed by Lomaestro and Bailie, 1993.

The mean (± SE) value of ratio between renal clearance of norfloxacin and that of endogenous creatinine following single administration of norfloxacin was 0.308 ± 0.050. Less than one ratio indicates back diffusion (Hasan, 1998). Thus, besides glomerular filtration, renal handling of norfloxacin involves back diffusion also.

Following the administration of norfloxacin, the influence of urine pH, diuresis and plasma concentration of norfloxacin on the renal clearance of norfloxacin was studied by regression/correlation analysis. The analysis showed a non significant correlation between the urine pH (r=-0.575), and diuresis (r= 0.322) and ratio of renal clearance of norfloxacin and renal clearance of endogenous creatinine. However, a significant (P<0.05) negative correlation (r=-0.885) between plasma concentration of norfloxacin and ratio of renal clearance of norfloxacin and renal clearance of endogenous creatinine attributed to the saturation of excretory mechanism at higher plasma levels which is indicative of involvement of active tubular secretion (Fig 1). Thus, renal handling of norfloxacin following its single intramuscular administration in sheep involves glomerular filtration, back diffusion and active tubular secretion. 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).

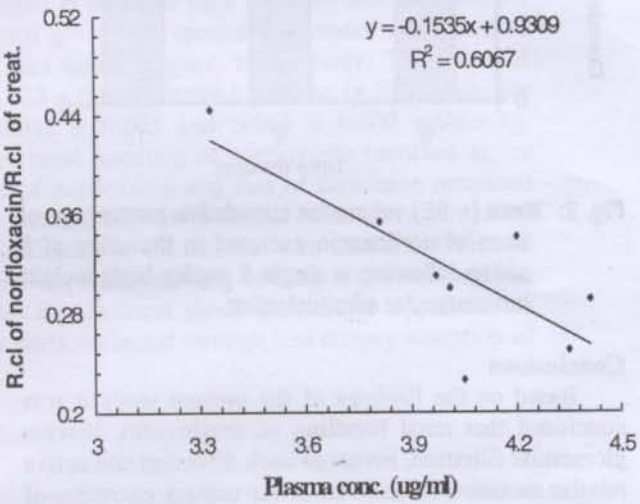

Fig. 1: Effect of plasma concentration of norfloxacin on its renal clearance following a single 5 mg/kg body weight intramuscular administration.

Urinary excretion

Mean (± SE) values of urinary excretion in terms of cumulative percent of dose of norfloxacin excreted in the urine at 6, 8 and 12 hours following intramuscular administrations have been presented in Fig 2. At 12 hours post drug administration, the value for the cumulative percent of dose of norfloxacin excreted in the urine was found to be 1.855 ± 0.045.

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 Montary *et el.* (1984), the urinary recovery of pefloxacin was 29% of the dose in mice, 37.8% in rats, 36.3% in dogs, 26.5% in monkeys and 58.9% in humans. Under current investigations, lower urinary excretion of norfloxacin in sheep may be evidenced by the respective results regarding its renal handling. These results show that regardless the involvement of active tubular secretion, the administered dose has also been absorbed at kidney tubular level through back diffusion. Moreover, in the present study, lower GFR in sheep may be responsible

for the lower urinary excretion of norfloxacin. Lower the GFR, least will be the urinary excretion of drug (Hasan, 1998).

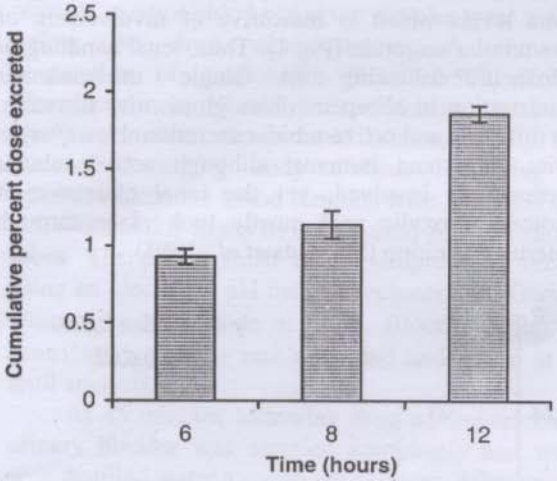

Fig. 2: Mean (± SE) values for cumulative percentage of dose of norfloxacin excreted in the urine of 8 sheep following a single 5 mg/kg body weight intramuscular administration.

Conclusions

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

REFERENCES

Afzal, G., M. Nawaz and B. Saleem, 1981. Renal clearance of endogenous creatinine and urea in sheep. Pakistan Vet. J., 1(3): 114-115.

Ahmad, M., 1983. Disposition kinetics of chloramphenicol in dogs and renal clearance of chloramphenicol in sheep. MSc Thesis, Univ. Agri., Faisalabad, Pakistan.

Akhtar, P., 1987. Renal clearance of endogenous and exogenous substances in domestic ruminiants during summer and winter seasons. PhD Thesis, Univ. Agri., Faisalabad, Pakistan.

Alvi, M. A., A. S. Hashmi and M. Nawaz, 1985. Renal clearance of endogenous and exogenous urea in sheep. Pakistan Vet. J., 5(3): 133-136.

Arret, B. D., D. Johnson and K. Amiel, 1971. Outline of details for microbiological assay of antibiotics. Pharmaceut. Sci., 60: 373-378.

Aslam, F., 1993. Creatinine, urea and kanamycin renal clearance in sheep. MSc Thesis, Univ. Agri., Faisalabad, Pakistan.

Bonsnes, R. M. and H. M. Taussky, 1945. The calorimetric determination of creatinine by the Jaffe reaction. J. Biol. Chem., 158: 581-591.

Hasan, I. J, 1998. Parmacokinetics, renal clearance and urinary excretion of kanamycin in domestic species. PhD Thesis, Univ. Agri., Faisalabad, Pakistan.

Iqbal, T., M. Nawaz, M. Ahmad and A. Mateen, 1986. Disposition kinetics and renal clearance of exogenous urea in sheep. Pakistan Vet. J., 6(4): 185-188.

Javed, I., M. Nawaz and F. H. Khan, 2003. Pharmacokinetics and optimal dosage of kanamycin in domestic ruminant species. Vet. Arhiv., 73: 323-331.

Lavy, E., G. Ziv and A. Glickman, 1995. Intravenous disposition kinetics, oral and intramuscular bioavailability and urinary excretion of norfloxacin nicotinate in donkeys. J. Vet. Pharmacol. Ther., 18: 101-107.

Lomaestro, B. M. and G. B. Bailie, 1993. Effect of multiple staggered doses of calcium on the bioavailability of norfloxacin. Ann. Pharmacother., 27 (11): 1325-1328.

Montary, G., Y. Goueffon and F. Roquet, 1984. Absorption, distribution, metabolic fate and elimination of pefloxacin mesylate in mice, rats, dogs, monkeys and humans. Antimicrob. Agents Chemother., 25(4): 463-472.

Muhammad, F., M. Nawaz and I. Javed, 2003. Disposition kinetics of kanamycin in mules. Vet. Arhiv, 73(4): 221-226.

Nawaz, M. and B. H. Shah, 1984. Renal clearance of endogenous creatinine and urea in sheep during summer and winter. Res. Vet. Sci., 36: 220-224.

Nawaz, M., 1994. Geonetical factors affecting biodisposition of drugs. Canad. J. Physiol. Pharmacol. Abst: p. 12.2.57, XII Int. Cong, Pharmacol., July 24-29, 1994, Montreal, Canada.

Nawaz, M., T. Iqbal and R. Nawaz, 1988. Geonetical considerations in disposition kinetic evaluation of chemotherapeutic agents. Vet. Pharmacol. Toxicol., Therapy in Food Producing Animals. 2: 260.

Shimada, J., T. Yamaji, Y. Ueda, H. Uchida, H. Kusajima and T. Irikura, 1983. Mechanism of renal excretion of AM-715, a new quinolonecaroxylic acid derivative, in rabbits, dogs and humans. Antimicrob. Agents Chemother., 23(1): 1-7.