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EFFECT OF DIPYRONE ON THE RENAL CLEARANCE AND URINARY EXCRETION OF NORFLOXACIN IN SHEEP

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ABSTRACT

The effect of dipyrone on the renal clearance and urinary excretion of norfloxacin was investigated in eight healthy female sheep. In each animal, after a single intramuscular administration of norfloxacin, 5 mg/kg body weight, a wash out period of seven days was given. Afterwards, norfloxacin was administered with dipyrone as concurrent intramuscular injection, 50 mg/kg body weight. Following a single and concurrent drug administration, blood and urine samples were collected at different time intervals and analyzed for norfloxacin and creatinine concentrations. The value of diuresis after single administration of norfloxacin was 0.023 ± 0.004 ml/min.kg, while following concurrent administration with dipyrone it was 0.014 ± 0.02 ml/min.kg. Mean values for renal clearance of creatinine following single and concurrent administration with dipyrone were 0.042 ± 0.005 and 0.027 ± 0.003 ml/min.kg, respectively, while respective values for renal clearance of norfloxacin were 0.013 ± 0.002 and 0.008 ± 0.001 ml/min.kg. The renal handling of norfloxacin involved active tubular secretion following its administration alone or with dipyrone. The ratio between the renal clearance of norfloxacin and dipyrone remained less than one after the administration of norfloxacin alone and concurrently with dipyrone, which was indicative of back diffusion of the drug. The mean values for the cumulative percent of dose norfloxacin excreted at 12 hours following administration of norfloxacin alone was 1.86 ± 0.05 and following its concurrent administration with dipyrone was 1.35 ± 0.05. Thus, it is evident that besides glomerular filtration, renal handling of norfloxacin also involved back diffusion and active tubular secretion. It was concluded that these mechanisms showed no change in sheep following norfloxacin administration with dipyrone. However, dipyrone reduced GFR inducing less urinary excretion of norfloxacin.

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

INTRODUCTION

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 (Katzung, 2001). Fluoroquinolones, being very common in veterinary medicine, are frequently used with the non-steroidal anti-inflammatory drugs (NSAIDs). So one of the factors that may jeopardize the successful therapy is the drug interaction when two or more drugs are used in combination. Many of NSAIDs increase the risk of interactions with other drugs (Niewinski and Juzwenko, 2000). Dipyrone belongs to pyrazolone group of NSAIDs and is most frequently used in combination with norfloxacin in various infectious diseases in animals.

The present study was designed to evaluate the effect of a non-steroid anti-inflammatory drug, dipyrone, on the renal clearance and urinary excretion of norfloxacin in sheep. It is hoped that proposed study will provide an insight into interaction, when norfloxacin is used concurrently with dipyrone.

MATERIALS AND METHODS

Experimental animals

Eight healthy adult female sheep with average weight of 42 Kg (34-48 Kg) were used in this study. All the sheep were maintained under similar environmental and managemental conditions at the University of Agriculture, Faisalabad, Pakistan. The animals were fed with seasonal green fodder and had free access to drinking water. Experiment was conducted during the months of June and July.

Renal clearance

In each animal, left jugular vein was cannulated 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. The external opening of the catheter was connected through rubber tubing to a urine-collecting reservoir in which all the voided urine was collected. In all animals control blood and urine samples were collected before the drug administration. Commercial preparations of norfloxacin (Norflox-50®, Kakasian Pharmaceuticals, Rawalpindi, Pakistan) and metamizole (Dipyrone, Lawrance Pharmaceuticals, Lahore, Pakistan) were used.

Norfloxacin was administered intramuscularly at the rate of 5 mg/kg body weight. After a wash out period of seven days, norfloxacin at the previous dosage level and dipyrone at the dosage level of 50 mg/Kg body weight were injected simultaneously. Following drugs administration, the blood samples were collected at 1, 1.5, 2 and 2.5 hours in heparinized plastic centrifuge tubes. After recording pH, blood samples were centrifuged, plasma was separated and stored at -4°C until analysis.

For renal clearance studies, urinary bladder was emptied completely 45 minutes following drug administration 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 concentration in plasma and urine samples was determined by a "Microbiological Assay" (Arret *et al*., 1971) according to disk agar diffusion method using sensitive microorganisms *E. coli* as test organism. The creatinine concentration in plasma and urine samples was determined by Jaffe-reaction using spectro-photometer (Spectronic 212, Bausch & Lomb, Germany) according to the method of Bonsnes and Taussky (1945). 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). The effect of dipyrone on the renal handling of norfloxacin following its administration alone and with dipyrone and 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

The urinary excretion of norfloxacin was investigated in the animals used for renal clearance studies. For this purpose, the urine samples were collected for the drug assay before, and at 6 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 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 administration of norfloxacin alone and concurrent administration with dipyrone was calculated.

Statistical analysis

The mean values (± SE) for each concentration in animlas given norfloxacin alone or with dipyrone were calculated. T-test was used to see the significance of difference between the two groups. 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 computer programme.

RESULTS AND DISCUSSION

Renal clearance

The mean (± SE) values for 32 sampling periods, four in each experiment, showing diuresis, blood and urine pH and the renal clearance of endogenous creatinine and norfloxacin in 8 sheep following administration of norfloxacin alone and its concurrent administration with dipyrone are presented in Table 1. The renal clearance of endogenous creatinine was measured as an index of glomerular filtration rate (GFR).

Table 1: Mean values (± SE) of diuresis, blood and urine pH, and renal clearance of endogenous creatinine and norfloxacin in sheep following administration of norfloxacin alone and with dipyrone

Parameters	Norfloxacin alone	Norflaxacin with dipyrone
Diuresis (ml/min.kg)	0.023 ± 0.004*	0.014 ± 0.002
Blood pH	7.51 ± 0.02*	7.27 ± 0.01
Urine pH	8.776 ± 0.03*	8.35 ± 0.03
Creatinine conc. (µg/ml)		
Plasma	3.50 ± 0.15^{NS}	3.99 ± 0.16
Urine	5.81 ± 0.16^{NS}	6.84 ± 0.21
Norfloxacin conc. (µg/ml)		
Plasma	4.17 ± 0.10^{NS}	4.02 ± 0.12
Urine	2.52 ± 0.04^{NS}	2.37 ± 0.05
Renal clearance (ml/min.kg)		
Creatinine	0.042 ± 0.005*	0.027 ± 0.003
Norfloxacin	0.013 ± 0.002*	0.008 ± 0.001
Norfloxacin cl: Creatinine cl ratio	0.308 ± 0.050^{NS}	0.314 ± 0.024

Each value represents the mean of four observations in four experimental periods in eight animals.
* = Significantly (P<0.05) different from respective value, NS = Non significant.

Mean renal clearance of creatinine in sheep following single administration of norfloxacin was 0.042 ± 0.005 ml/min.kg which reduced to 0.027 ± 0.003 ml/min.kg following concurrent administration of norfloxacin with dipyrone rendering the reduction as 36%. However, the respective value for the renal clearance of norfloxacin changed from 0.013 ± 0.002

ml/min.kg to 0.008 ± 0.001ml/min.kg, showing 38% reduction.

In the present study, concurrent administration of norfloxacin with dipyrone decreased renal clearance of norfloxacin, as well as renal clearance of endogenous creatinine. In earlier studies fenbufen after its concurrent administration reduced the renal clearance of ciprofloxacin by 20% (Naora *et al*., 1990). Antacids reduced the clearance of norfloxacin by 15% (Nix *et al*., 1991). A 19% reduction in the renal clearance of furosemide was observed after its concurrent administration with metamizole in healthy volunteers (Rosenkranz *et al*., 1992). A significant decrease in theophylline clearance was observed when given concurrently with ciprofloxacin in man (Hulsiz and Miller, 1990). As the renal clearance of endogenous creatinine is an index of glomerular filtration rate, an obvious decrease in the renal clearance of endogenous creatinine may be indicative of lower GFR for norfloxacin following its concurrent administration with dipyrone. Thus, after its concurrent administration, dipyrone reduced renal clearance of norfloxacin.

The influence of urine pH and diuresis on the renal clearance of norfloxacin following the administration of norfloxacin alone and with dipyrone showed a non significant correlation between the urine pH and diuresis and ratio of renal clearance of norfloxacin and renal clearance of endogenous creatinine. However, a significant ($P<0.05$) negative correlation between plasma concentration of norfloxacin and ratio of renal clearance of norfloxacin and renal clearance of endogenous creatinine following norfloxacin administration alone and with dipyrone was attributed to the saturation of excretory mechanisms at higher plasma levels which is indicative of involvement of active tubular secretion (Figs. 1 and 2). The ratio

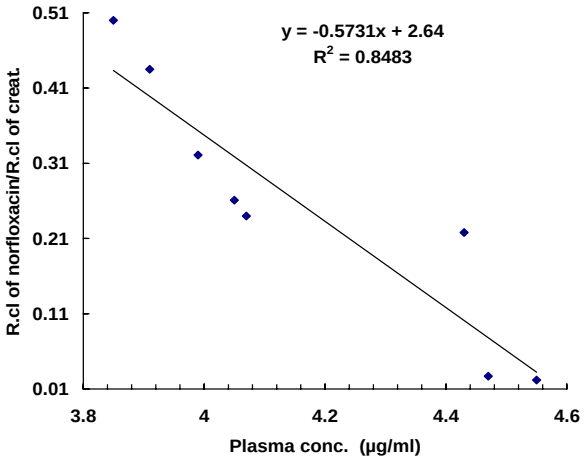

Fig. 1: Effect of plasma concentration of norfloxacin on its renal clearance following single administration. Each data point represents the mean of four observations in four experimental periods in 8 animals.

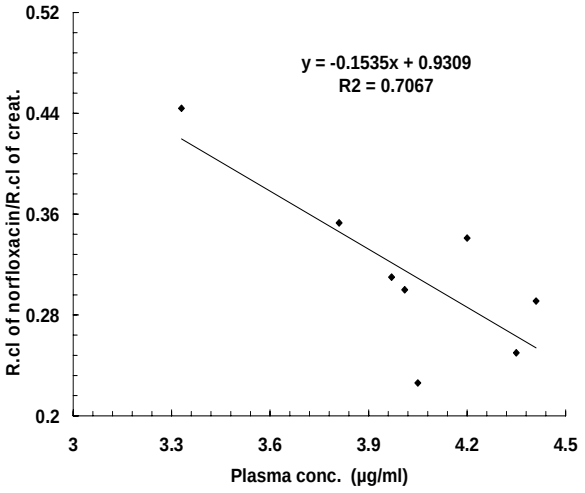

Fig. 2: Effect of plasma concentration of norfloxacin on its renal clearance following concurrent administration with dipyrone. Each data point represents the mean of four observations in four experimental periods in 8 animals

between the renal clearance of norfloxacin and dipyrone remained less than one after the administration of norfloxacin alone and concurrently with dipyrone (Table1), which indicates back diffusion.

It appears that following administration of norfloxacin alone, the renal handling of norfloxacin, besides glomerular filtration, also involved active tubular secretion and back diffusion. However, after concurrent administration, dipyrone reduced the GFR of norfloxacin inducing its less renal clearance but showed no change in mechanisms of back diffusion and active tubular secretion. Thus, dipyrone, except reduction in GFR, showed no effect on the renal handling of norfloxacin.

Urinary excretion

Mean (± SE) values of urinary excretion in terms of cumulative percent of dose of norfloxacin excreted in the urine at 6 and 12 hours following both single and concurrent administrations have been compared in Fig. 3. Mean values for the cumulative percent of dose of norfloxacin excreted at 12 hours following administration of norfloxacin alone was 1.855 ± 0.045 versus 1.350 ± 0.054 following its concurrent administration with dipyrone. These results show that the cumulative percent of dose excreted at 12 hours in urine of sheep decreased from 1.855 ± 0.045 to 1.350 ± 0.054 following its concurrent administration with dipyrone. Both the values are significantly ($P<0.05$) different, showing about 20% reduction. In earlier studies, mean percentage of sparfloxacin dose recovered in urine was significantly lower following its

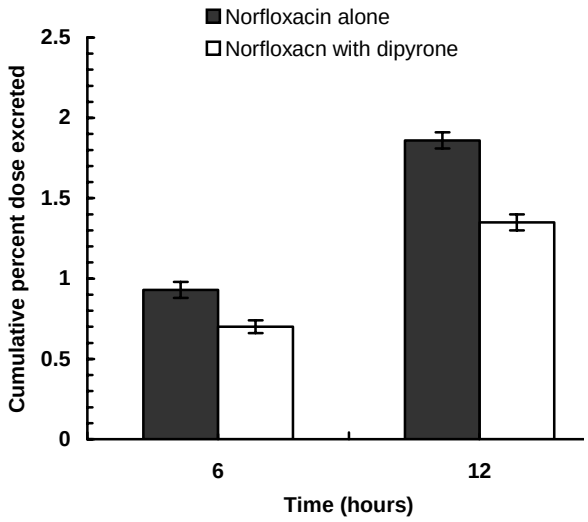

Fig. 3: Mean (± SE) values for cumulative percentage of dose of norfloxacin excreted in urine of 8 sheep following its single and concurrent administration with dipyrone.

administration in combination with sucralfate (Marika *et al*., 1999). The percent urine recovery of norfloxacin was reduced when it was administered concurrently with sucralfate (Salvinaz *et al*., 1989). Fenbufen significantly reduced the cumulative urinary excretion of quinolones (Naora *et al*., 1990).

Under current investigations, lower urinary excretion of norfloxacin in sheep following its concurrent administration with dipyrone 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, following its concurrent administration with norfloxacin, dipyrone reduced GFR. Lower the GFR, least will be the urinary excretion of drug (Hasan, 1998). Furthermore, the pH of urine although remained alkaline, yet decreased after concurrent administration of norfloxacin and dipyrone (Table1). At comparatively less alkaline urinary pH, the ionized moiety of the weakly acidic drug like norfloxacin decreases giving way to unionized moiety suitable for absorption at kidney tubular level.

Conclusion

Based on the findings of the present study, it can be concluded that renal handling of norfloxacin besides glomerular filtration involved back diffusion and active tubular secretion. However, no effect of dipyrone was observed on these mechanisms in sheep.

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