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PHARMACOKINETIC STUDIES OF OFLOXACIN FOLLOWING ORAL ADMINISTRATION IN GOATS

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ABSTRACT
The pharmacokinetics of Ofloxacin was estimated following single oral administration in goats to compute a rational dosage regimen. HPLC and non-compartmental open model was used to determine the plasma Ofloxacin concentration and to calculate kinetic parameters resepectively. The therapeutic concentration was achieved in 45 min and maintained up to 8 h. The C_{max}, t_{max}, AUC and AUMC values were 1.168 ± 0.07 mg/ mL, 5.17 ± 0.72 h, 11.41 ± 1.13 m/h/mL and 221.11 ± 38.44 mg/h/ml respectively. The mean absorption half life ($t_{1/2\ ka}$) and mean absorption time (MAT) of 5.40 ± 0.78 h and 7.79 ± 1.13 h respectively. In goats, Ofloxacin showed poor bioavailability with a value of 24.14 ± 1.70 % and slow elimination with mean residence time (MRT) of 16.05 ± 1.19 h. Keeping in view of the above kinetic parameters of Ofloxacin, a rational loading dose of 21 mg/kg followed by the maintenance dose of 13 mg/kg at 24 h dosing interval by oral route can recommended in goats.

INTRODUCTION

Injudicious and wide spread use of antibiotics is one of the major cause of growing antibacterial resistance in both human as well as animals which has lead to advent of newer antibacterial to combat microorganism that were otherwise susceptible to older antibacterials. Ofloxacin, a broad spectrum fluoroquinolone antimicrobial, widely used in the treatment of septicemia, respiratory tract, urinary tract, skin, soft tissues, bone and joint infections in humans (Beermann *et al.*,1984). Pharmacokinetic properties of ofloxacin have been investigated in cattle (Gaur *et al.*, 2004), chicken (Kalaiselvi *et al.*, 2006) and pigs (Son *et al.*, 2000). Pharmacokinetic data of Ofloxacin in goat are inadequate to warrant its effective clinical use in goats (*Capra hircus*). In the light of the above context, the study of pharmacokinetics of ofloxacin was taken up to investigate the pharmacokinetic pattern of the drug and to calculate a rational oral dosage regimen in goat.

MATERIALS AND METHODS

The method of Teja Isavadharm *et al.* (1991) was followed with some modification for quantitative determination of plasma ofloxacin concentration by HPLC.

The study was conducted on six clinically healthy male goats (*Capra hircus*) of Assam of age between 8-18 months old and weighing 10-16 kg. The animals were stall fed and water was provided *ad libitum*. The pure standard (97.5%) and commercial preparation (Zanocin tablets -200 mg) of the drug was supplied by Ranbaxy India Limited. The drug was administered orally at the dose rate of 5mg/kg body weight. Blood sample (3 ml) was collected by jugular venipuncture into heparinized test tubes at 0, 5, 15, 30, 45, 60 (1 h), 90 (1.5 h), 120 (2 h), 180 (3 h), 240 (4 h), 360 (6 h), 480 (8 h), 600 (10 h), 720 (12 h), 1440 (24 h), 2160 (36 h), 2880 (48 h), 4320 (72 h) and 5760 (96 h) min. Plasma was harvested by centrifugation at 3000 rpm for 15 min and stored at -20ºC until assayed for ofloxacin. The analysis for ofloxacin in plasma was performed on a HPLC system (Perkin Elmer, USA) consisting of a binary LC pump, a diode arry detector, a LC-100 laboratory computing integrator and a μ Bondapac C_{18} column (Waters, USA, 30 mm x 3.9 mm ID and 10 μm particle size). The mobile phase consists of 0.1M phosphoric acid (adjusted to pH 2.5 with a solution of 45% potassium hydroxide) and acetonitrile mixed in a ratio of 75: 25 (v/v). The flow rate of mobile phase was 1.2 ml/min and the eluent was monitored in Diode array detector. The chromatograms were integrated on the LC-100 laboratory computing integrator. Plasma samples were subjected to liquid-phase extraction. To 1 ml of plasma, 1 mL of methanol was added, mixed by vortexing for 20 seconds and then placed on ice for 15 min to enhance precipitation. It was centrifuged at 15,600 g for 10 min and the supernatant (750 μL) was transferred to another tube. Dichloromethane (6mL) was added and the contents were mixed by vortexing for 20 seconds followed by centrifugation at 1000 g for 10 min. The organic and aqueous phases formed were separated by using phase separator filter

paper. After discarding the aqueous phase, the organic phase was transferred to a clean siliconized tube and evaporated to dryness at 40ºC. The residue was then reconstituted in mobile phase (500 μL) and 20 μL was injected into column. The standard curve was prepared by spiking blank plasma with standard parent compound at different concentration ranging from 0.025 to 20 g/mL (1.25 to 20 μg/mL) and extracted by liquid phase extraction as described above. The plasma concentrations of ofloxacin in the samples were determined by comparing the detector response for the drug in the sample with the corresponding standards (Fig. 1). Extraction recovery was determined by comparing the peak area of an extracted spiked sample with the peak area of direct injection of the mobile phase containing same concentration of pure drug. The extraction recovery and limit of quantification of ofloxacin in plasma was found to be 99.2% and 0.01 mg/L respectively.

Pharmacokinetic analysis

The concentration of ofloxacin in plasma were plotted on a semi-logarithmic scale as a function of time and the pharmacokinetic parameters were calculated for each animal by using statistical moments approach (Riviere, 2011). The dosage regimen was computed by the method of Wartak (1983) and Benet *et al.* (1996). To maintain the desired therapeutic concentration in plasma, the loading or priming and maintenance doses at suitable dosing interval were calculated by using the following formulae

$$\text{Maintenance dose} = \frac{Css \times V \times T}{F \times 1.44 \times t_{1/2}}$$

$$\text{Loading dose} = \frac{\text{Maintenance dose}}{1 - e^{-kT}}$$

(C_{ss} is average steady state plasma concentration, V is apparent volume of distribution, T is dosing interval, F is bioavailability and $t_{1/2}$ is half-life)

RESULTS AND DISCUSSION

Plasma ofloxacin concentrations at various time intervals following oral administration in goats were given in Table 1 and Fig 2. The data do not follow any compartmental model and hence non compartmental method of analysis was carried out. Various kinetic variables were computed and listed in Table 2.

Following oral administration of ofloxacin (5 mg/kg), the minimum inhibitory concentration (MIC 0.5 mg/mL) was observed at 45 min and maintained up to 8 h in goat. The mean maximum blood concentration (C_{max}) of 1.16 ± 0.07 μg/mL was achieved at 5.16 ± 0.72 h (t_{max}). Monk and Campoli

Table 1: Plasma concentration (μg /ml) of ofloxacin and its mean ± in goat following single oral dose of 5 mg /kg body weight (n = 6, G1, G2 etc. means Goat1, Goat 2 etc.)

Time (hr)	G1	G2	G3	G4	G5	G6	Mean+SE
0.08	0.034	0	0	0	0	0	0.006 ± 0.01
0.25	0.089	0	0	0.012	0.016	0	0.020 ± 0.01
0.5	0.08	0.007	0.039	0.03	0.047	0.06	0.044 ± 0.01
0.75	0.735	0.116	0.031	0.581	0.897	0.713	0.512 ± 0.15
1	0.386	0.143	0.278	0.582	1.744	0.409	0.590 ± 0.24
1.5	0.711	1.252	0.181	0.318	1.16	0.688	0.718 ± 0.18
2	0.742	0.286	0.159	0.627	1.396	0.72	0.655 ± 0.18
3	0.41	0.093	0.098	0.628	0.484	0.287	0.333 ± 0.09
4	0.297	0.15	0.051	0.207	0.066	0.388	0.193 ± 0.05
6	0.309	1.385	0.161	0.788	0.024	0.275	0.490 ± 0.21
8	0.488	0.049	0.195	0.971	0.816	0.465	0.497 ± 0.14
10	0.451	0.036	1.073	0.163	0.201	0.429	0.392 ± 0.15
12	0.079	0.024	1.443	0.265	0.227	0.559	0.433 ± 0.22
24	0.59	0.025	0.177	0.235	0.029	0.127	0.197 ± 0.09
36	0.56	0.014	0.167	0.089	0.028	0.104	0.160 ± 0.08
48	0.032	0	0.169	0.032	0.016	0.034	0.047 ± 0.02
72	0.025	0	0.096	0.014	0.013	0.034	0.034 ± 0.01
96	0	0	0.075	0	0	0	0.013 ± 0.01

Table 2: Pharmacokinetic determination of ofloxacin in goats following single oral dose of 5mg/kg body weight.

Pharmacokinetic determinants	Unit	G1	G2	G3	G4	G5	G6	Mean ± SE
AUC	μg. h/mL	7.46	4.73	23.93	12.27	7.95	12.13	11.41 ± 1.13
AUMC	μg. h2/mL	122.86	32.92	671.11	201.15	87.37	211.28	221.11 ± 38.44
MRT_0	h	16.48	6.96	28.05	16.40	10.99	17.41	16.04 ± 1.19
$t_{1/2ka}$	h	9.43	12.38	2.00	0.23	5.56	2.83	5.40 ± 0.78
Ka	/h	0.07	0.06	0.35	3.06	0.13	0.25	0.651 ± 0.20
C_{max}	μg /mL	0.74	1.39	1.44	0.97	1.74	0.72	1.16 ± 0.07
t_{max}	h	2.00	6.00	12.00	8.00	1.00	2.00	5.16 ± 0.72
F	%	10.37	25.27	15.09	27.77	27.19	39.15	24.14 ± 1.70
MAT_0	h	13.61	17.86	2.88	0.33	8.03	4.08	7.79 ± 1.13
Cl_0	ml/h/kg	670.69	1056.64	208.95	407.60	628.93	412.10	564.15 ± 48.98

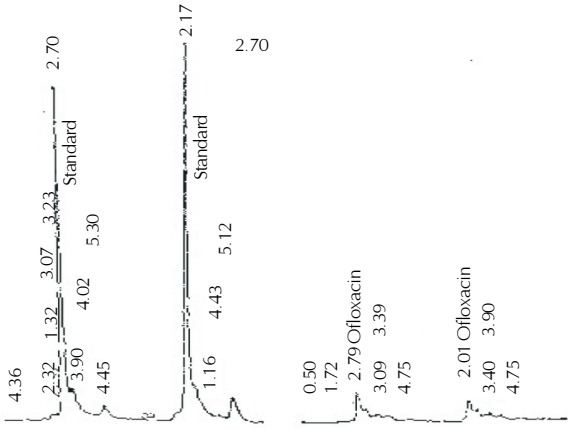

Figure 1: Representative chromatograms of ofloxacin in goat plasma

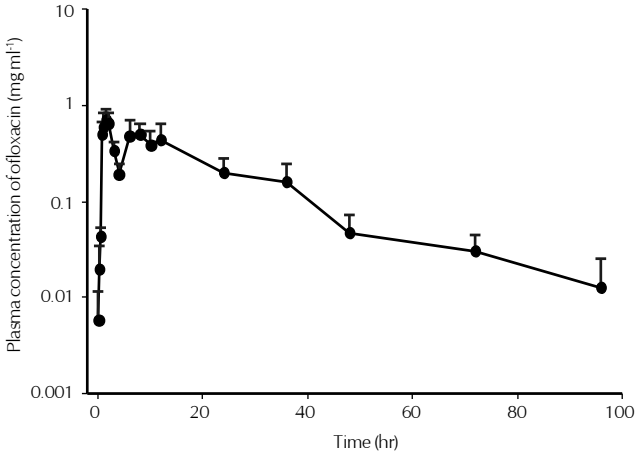

Figure 2: Plasma ofloxacin concentrations at various time intervals following oral administration in goats

(1987) reported mean range of C_{max} and t_{max} in human after a single oral dose of ofloxacin (400 mg) were 4.0-5.6 mg/ml and 0.7-1.4 h, respectively. Low C_{max} value in goat might be due to the low bioavailability of the drug as compared to man. The absorption $t_{1/2\,ka}$ (5.40 ± 0.78 h) and MAT_0 (7.79 ± 1.13 h) and MRT (16.41 ± 1.19 h) indicated slow absorption rate and elimination of the Ofloxacin in goat. Comparative $t_{1/2ka}$ in human after a single oral dose of 200 mg was 5.7-7.0 h (Monk and Campoli,1987) and 4.82 h following single oral dose of ofloxacin (10 mg/kg) in chicken (Liu and Fung, 1997). The AUC value observed in present study was 11.41 ± 1.13 mg/h/mL which is in census with the reported AUC value in buffalo is 12.40 mg h /mL after a single oral dose of 5 mg/kg (Kumar *et. al.*, 2008). The lower value of AUC and bioavailability in ruminants might be due to degradation of the parent molecule in ruminal environment. The bioavailability (F) of ofloxacin in systemic circulation following oral administration (5 mg/kg) in goat was found to be 24.14 ± 1.70 % as compared to 85-95% in rabbits (Ahmed *et al.*, 2008).

On the basis of kinetic analysis of the data and keeping in view of the minimum concentration of the drug a priming / Loading dose of 21 mg/kg or 18 mg/kg followed by a corresponding maintenance dose of 13 mg/kg or 8 mg/kg at 24 hr or 12 hr respectively would maintain a MIC of e" 0.5 mg/mL required for most pathogenic microorganism and this dosage regimen would maintain a steady concentration of Ofloxacin (C_{ss}) between 1.5 mg/ mL and 0.5 mg/ mL receptively (average C_{ss} 1 mg/ mL) in between the dosing interval.

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