

Pharmacokinetics, tissue residue and plasma protein binding of ofloxacin in goats

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Ofloxacin was administered to six male goats intravenously (5 mg/kg) to determine its kinetic behavior, tissue residue, *in vitro* plasma protein binding and to compute a rational dosage regimen. The concentration of ofloxacin in plasma and tissue samples collected at prescheduled time were estimated by using HPLC. The pharmacokinetic parameters were determined by non-compartmental model and plasma protein binding was estimated by equilibrium dialysis technique. The therapeutic concentration ($\geq 0.5 \mu\text{g/ml}$) was maintained up to 36 h and the initial concentration at 2.5 min ($14.76 \pm 0.47 \mu\text{g/ml}$) declined to $0.05 \pm 0.03 \mu\text{g/ml}$ at 96 h with a secondary peak ($0.64 \pm 0.15 \mu\text{g/ml}$) at 24 h. The mean AUC, AUMC, $t_{1/2}$, MRT, CI and V_d were calculated to be $58.94 \pm 19.43 \mu\text{g} \cdot \text{h/ml}$, $1539.57 \pm 724.69 \mu\text{g} \cdot \text{h}^2/\text{ml}$, $15.58 \pm 1.87 \text{ h}$, $22.46 \pm 2.71 \text{ h}$, $135.60 \pm 31.12 \text{ ml/h/kg}$ and $2.85 \pm 0.74 \text{ L/kg}$ respectively. Significantly high concentration of drug was detected in different tissues after 24 h of intravenous dosing of 5 mg/kg, at 24 h interval for 5 days. The *in vitro* plasma protein binding of ofloxacin was found to be $15.28 \pm 0.94\%$. Based on these kinetic parameters, a loading dose of 5 mg/kg followed by the maintenance dose of 3 mg/kg at 24 h dosing interval by intravenous route is recommended.

Key words: Pharmacokinetics, Ofloxacin, dosage, goats

Introduction

One of the ominous trend in the field of antimicrobial therapy over the past decades has been the increasing pace of development of antimicrobial resistance in bacterial pathogens and emergence of new resistant strains. Fluoroquinolones have emerged as a novel class of antimicrobial agents against some troublesome resistant pathogens. Ofloxacin, a new generation fluoroquinolone, have broad spectrum of activity against variety of gram positive and gram negative bacteria and some anaerobes [13]. Pharmacokinetic studies of ofloxacin have been reported in dog [19], rabbit [12], mice [4], rat [6], chicken [9], and human [7]. Detailed pharmacokinetic data of this antimicrobial agent is lacking in goat. Therefore, the objective of the present study was to investigate the pharmacokinetic pattern, tissue residue and plasma protein binding of the drug following single intravenous administration in goat. The pharmacokinetic data obtained was applied for computing optimal dosage regimen, which will promote rational use of the drug in this species, while reducing the risk of drug related toxicity.

Materials and Methods

Animals

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 kept for 2 weeks before commencement of the experiment for acclimatization. During the experimental period the animals were maintained on concentrate feed and free grazing. Water was provided *ad libitum*.

Drugs

The pure standard of ofloxacin and injectable commercial preparation, Zanocin infusion (200 mg/100 ml of distilled water) was manufactured by Ranbaxy Laboratories Ltd., India. The drug was administered by intravenous administration into the jugular vein with 5 mg/kg body weight.

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For tissue residue study, ofloxacin (5 mg/kg body weight) was administered intravenously at 24 h interval for 5 consecutive days.

Sample collection

Blood sample (3 ml) were collected into heparinized test tubes by jugular venipuncture. The samples prior to and after administration of the drugs were collected at 0, 2.5, 5, 10, 20, 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 -20°C until assayed for ofloxacin. For studying tissue residue, four animals were sacrificed by decapitation after the last dose a 5 days dosing schedule and a representative sample (1 g) of different tissues viz. liver, kidney, heart, lung, brain, fat and skeletal muscle were collected. Tissues were accurately weighed, cut into small pieces, homogenized with normal saline solution and stored at 20°C until analyzed.

In vitro plasma binding was determined by equilibrium dialysis technique [8]. Plasma concentration of ofloxacin, i.e. 1.25, 2.5, and $5\ \mu\text{g}/\text{ml}$ was dialyzed (pore size 4°A) for 24 hours at 37°C with phosphate buffer (0.2 M; pH 7.4).

Analytical method

For quantitative determination of ofloxacin in plasma, the HPLC method of Teja-Isavadharm *et al.* [17] was followed with some modification.

The analysis for ofloxacin in plasma was performed on a HPLC system (Perkin Elmer, USA) consisting of a binary LC pump, a diode array detector, a LC-100 laboratory computing integrator and a μ Bondapac C_{18} column (Waters, USA, $30\ \text{mm} \times 3.9\ \text{mm}$ ID and $10\ \mu\text{m}$ particle size).

The mobile phase consist of 0.1 M 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 chromatogram 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 $15,600\ \text{g}$ for 10 min and the supernatant ($750\ \mu\text{l}$) was transferred to another tube. Dichloromethane (6 ml) was added and the content were mixed by vortexing for 20 seconds followed by centrifugation at $1000\ \text{g}$ for 10 min. The organic and aqueous phase 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 40°C . The residue was then reconstituted in mobile phase ($500\ \mu\text{l}$) and was injected into

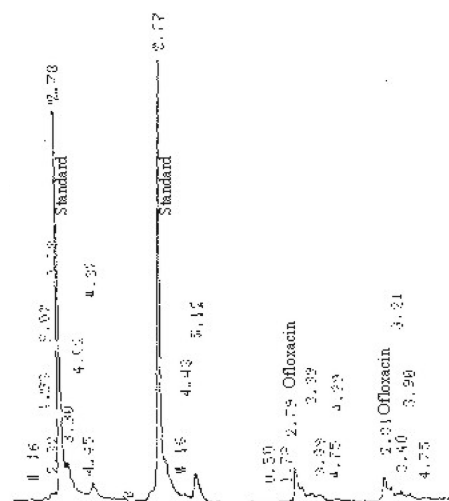


Fig. 1. Representative chromatograms of ofloxacin in goat plasma.

column.

The standard curve was prepared by spiking blank plasma with standard parent compound at different concentration ranging from 0.025 to $20\ \mu\text{g}/\text{ml}$ and extracted by liquid phase extraction as described above. The plasma concentration of ofloxacin in the samples were determined by comparing the detector response for the drug in the sample with the corresponding standards (Fig. 1).

The homogenized tissue samples were subjected to liquid phase extraction and estimated by using chromatographic conditions as described above for plasma samples. Ofloxacin concentrations in the tissue samples were determined by comparing with the corresponding tissue standards.

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 $0.01\ \text{mg}/\text{L}$ respectively. The extraction recovery and the limit of quantification for all the tissue was near 100% and $10\ \mu\text{g}/\text{ml}$ 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 [11]. The dosage regimen was computed by the method of Wartak [18] and Benet *et al.* [2]. 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:

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

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

Where,

C_{ss} = Average steady state plasma concentration.

V = Apparent volume of distribution.

T = Dosing interval.

F = Bioavailability.

t_{1/2} = Half-life.

K = Overall elimination rate constant.

Table 1. Plasma concentration of ofloxacin (µg/ml) in goats following a single intravenous dose of 5 mg/kg body weight (n = 6).

Time after ofloxacin Administration (min)	Mean ± SE	Range
2.5	14.76 ± 0.47	12.21 - 19.88
5	10.30 ± 0.05	7.15 - 15.64
10	9.03 ± 0.42	4.97 - 12.73
20	7.48 ± 0.34	4.12 - 10.08
30	6.56 ± 0.28	4.41 - 8.58
45	4.79 ± 0.30	2.24 - 6.75
60 (1 h)	5.32 ± 0.26	2.40 - 6.84
90 (1.5 h)	4.95 ± 0.28	1.81 - 6.50
120 (2 h)	3.94 ± 0.25	1.99 - 5.61
180 (3 h)	2.92 ± 0.42	0.19 - 6.87
240 (4 h)	1.71 ± 0.24	0.16 - 4.41
360 (6 h)	0.94 ± 0.17	0.13 - 2.91
480 (8 h)	0.87 ± 0.15	0.07 - 2.59
600 (10 h)	0.62 ± 0.14	0.03 - 2.32
720 (12 h)	0.53 ± 0.13	0.02 - 2.14
1440 (24 h)	0.64 ± 0.15	0.05 - 2.31
2160 (36 h)	0.62 ± 0.14	0.06 - 2.04
2880 (48 h)	0.44 ± 0.07	0.09 - 1.21
4320 (72 h)	0.35 ± 0.03	0.11 - 0.99
5760 (96 h)	0.05 ± 0.03	0.02 - 0.19*

*n = 4, Not detected in two animals.

Results

Plasma concentration of ofloxacin at various time intervals following single intravenous administration (5 mg/kg) are given in Table 1 and its semilogarithmic graphical representation is presented in Fig. 2. The mean plasma concentration at 2.5 min was 14.76 ± 0.47 µg/ml which declined to 0.50 ± 0.03 µg/ml at 96 h with a secondary peak of 0.64 ± 0.15 µg/ml at 24 h. The therapeutic concentration (≥0.5 µg/ml) was maintained up to 36 h post-administration of the drug. The values of various pharmacokinetic parameters are presented in Table 2. The concentration of ofloxacin in different tissues were found to be in the order of liver > kidney > lung > skeletal muscle > heart > fat > brain (Table 3). The percent plasma protein binding of ofloxacin at different plasma concentrations of 1.25, 2.5 and 5 µg/ml are to the extent of 26.78 ± 1.51, 10.14 ± 0.27 and 8.92 ± 1.06% respectively, with an overall mean of 15.28 ± 0.94% (Table 4).

Discussion

Following single intravenous administration, ofloxacin

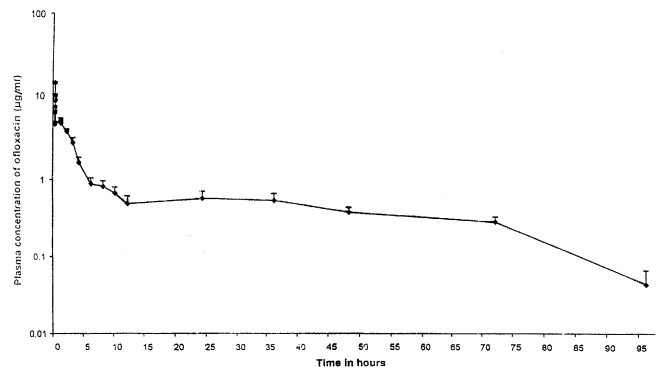


Fig. 2. Graphical representation of mean plasma concentration of ofloxacin following single I.V. dose of 5 mg/kg body weight.

Table 2. Pharmacokinetic determinants of ofloxacin in goats following single intravenous dose of 5 mg/kg body weight (n = 6)

PK Determinants	Unit	G ₁	G ₂	G ₃	G ₄	G ₅	G ₆	Mean ± SE
AUC	µg · h/ml	71.91	18.73	158.59	44.17	29.24	30.99	58.94 ± 19.43
AUMC	µg · h ² /ml	2163.9	460.72	4904.72	738.7	1556.04	413.25	1539.57 ± 724.69
MRT	h	30.09	24.81	30.98	16.73	19.02	13.34	22.46 ± 2.71
t _{1/2}	h	20.85	17.20	21.81	11.59	13.18	9.24	15.58 ± 1.87
K	h ⁻¹	0.03	0.04	0.03	0.06	0.05	0.07	0.05 ± 0.01
Cl	ml/h/kg	69.53	266.98	31.53	113.21	171.01	161.35	135.60 ± 31.12
V _d	L/kg	2.11	6.67	0.99	1.92	3.23	2.18	2.85 ± 0.74
V _{dss}	L/kg	2.09	6.62	0.98	1.89	3.25	2.15	2.83 ± 0.74

G₁-G₆ = Number of goats.

AUC = Total area under the plasma concentration versus time curve; AUMC = Area under the first moment curve; MRT = Mean residence time; t_{1/2} = Elimination half life; K = Apparent overall first order elimination rate constant; Cl = Total body clearance; V_d = Apparent volume of distribution; V_{dss} = Steady state volume of distribution.

Table 3. Ofloxacin concentration ($\mu\text{g/g}$) in different tissues after 24 h of intravenous dosing of 5 mg/kg body weight, at 24 h interval for 5 days (n = 4)

Tissues	Concentration of ofloxacin in tissues ($\mu\text{g/g}$)				Mean \pm SE
	I	II	III	IV	
Liver	4.01	4.81	1.01	6.01	3.96 \pm 0.92
Kidney	3.26	3.34	0.48	3.91	2.75 \pm 0.67
Lung	1.38	2.16	1.65	2.59	1.95 \pm 0.23
Skeletal muscle	1.52	1.26	1.65	2.59	1.54 \pm 0.09
Heart	0.47	0.58	0.72	0.57	0.59 \pm 0.05
Fat	0.46	0.99	0.08	0.56	0.52 \pm 0.16
Brain	0.10	1.05	0.46	0.12	0.43 \pm 0.19

Table 4. *In Vitro* plasma protein binding of ofloxacin in goat

Ofloxacin Concentration in plasma ($\mu\text{g/ml}$)	Percent protein binding			Mean \pm SE
	I	II	III	
1.25	31.51	26.35	22.48	26.78 \pm 1.51
2.5	10.12	9.33	10.97	10.14 \pm 0.27
5	8.09	8.30	10.37	8.92 \pm 1.06

Overall mean = 15.28 \pm 0.94

was detected in plasma up to 96 h and the mean plasma-ofloxacin concentration time profile showed a secondary peak (0.64 \pm 0.15 $\mu\text{g/ml}$) at 24 h. The appearance of the secondary peak seems to be due to enterohepatic circulation of the drug. The enterohepatic circulation of the drug that is extensively cleared by into the bile may produce secondary peak in plasma level time profile [1]. Similar time course of ofloxacin (5 mg/kg) was reported in sheep [16]. The analysis of the semi-logarithmic plasma-ofloxacin time profile curve revealed that it could best be analyzed by non-compartmental model. More and more investigators and clinicians who use pharmacokinetic are turning to non-compartmental approaches, since pharmacokinetic analysis based on compartmental models can lead to un-reconcilable difficulties.

The therapeutic concentration of ofloxacin ($\text{MIC}_{90} \geq 0.5 \mu\text{g/ml}$) was maintained up to 36 h, which is reflected by larger values of elimination half-life (15.58 \pm 1.87 h) and its analogous parameter, MRT (22.46 \pm 2.71 h). A relatively shorter half-life has been reported in man (5.4 h) [3], rabbit (1.5-1.9 h) [12] and in chicken (4.82 h) [9]. The longer residence of the drug in the body was further supported by high value of AUC (58.94 \pm 19.43 $\mu\text{g} \cdot \text{h/ml}$) and low clearance rate Cl (135.60 \pm 31.12 ml/h/kg). Enterohepatic recycling is often associated with multiple peaks and a longer apparent half-life in a plasma concentration-time profile [15]. The reported AUC of ofloxacin in rabbit [12], human [3], sheep [16] and in chicken [9] have been 37.09, 14.0, 418.40 and 47.08 $\mu\text{g} \cdot \text{h/ml}$ respectively. The mean

volume of distribution (V_d) of ofloxacin in the present study was found to be 2.85 \pm 0.74 L/kg indicating wide tissue distribution. The reported values of V_d in man [10] and in sheep [16] have been 2.4 3.5 L/kg and 1.61 L/kg respectively.

In tissue residue study, high concentration of ofloxacin was detected in liver (3.96 \pm 0.92 $\mu\text{g/g}$), kidney (2.75 \pm 0.67 $\mu\text{g/g}$), lung (1.95 \pm 0.23 $\mu\text{g/g}$) and in skeletal muscles (1.54 \pm 0.09 $\mu\text{g/g}$) as compared to heart (0.59 \pm 0.05 $\mu\text{g/g}$), fat (0.52 \pm 0.16 $\mu\text{g/g}$) and brain (0.43 \pm 0.19 $\mu\text{g/g}$). In human volunteers, high concentration of ofloxacin was reported to be achieved in liver, gall bladder, muscle (about 1.4 times higher), while in subcutaneous fat and in skin it was 50% or less than that of serum. Result of the *in-vitro* plasma protein binding showed low protein binding (15.28 \pm 0.94%), suggesting that conditions which alter protein binding would not influence the drugs pharmacokinetics. Ofloxacin has been reported to be about 20 % bound to serum in man [14].

Based on the pharmacokinetic parameters obtained in the present study, the dosage regimen of ofloxacin at 24 h interval was computed for intravenous administration in goat. An initial loading dose of 5 mg/kg followed by maintenance dose of 3 mg/kg at 24 h dosing interval is recommended. The suggested dosage regimen is expected to maintain the desired therapeutic concentration of the drug ($\geq 0.5 \mu\text{g/ml}$) in plasma for the treatment of diseases caused by susceptible bacteria. The dosage will maintain the steady state concentration of the drug in the range of 1.5 $\mu\text{g/ml}$ ($C_{ss \max}$) and 0.5 $\mu\text{g/ml}$ ($C_{ss \min}$) with an average C_{ss} of 1 $\mu\text{g/ml}$ between the dosing interval.

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