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PHARMACOKINETICS AND DOSAGE REGIMEN OF MOXIFLOXACIN FOLLOWING SINGLE INTRAVENOUS ADMINISTRATION IN SHEEP

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The study was conducted using six healthy Patanwadi female sheep. High Performance Liquid Chromatography (HPLC) with fluorescence detector assay method was employed to estimate the moxifloxacin plasma concentration versus time data after intravenous administrations. The mean plasma moxifloxacin level of 10.249 μg.ml^{-1} was observed at 5 min following single dose intravenous administration. The $t_{½K(a)}$, $t_{½\beta}$, $Vd_{(area)}$, $Vd_{(ss)}$, Cl_B and AUC were 0.660 h, 3.908 h, 2.512 L.kg^{-1}, 1.252 L.kg^{-1}, 0.445 L.h.kg^{-1} and 11.254 μg.h.ml^{-1} respectively. For treating systemic infection, a priming dose 5.5 mg.kg^{-1} followed by maintenance dose 5.0 mg.kg^{-1} at 12 h interval. Present study reveals that moxifloxacin concentration remains in the body of sheep for up to 24 h above MIC level after i.v. routes of administration with good value of $Vd_{(area)}$. This indicates rational therapeutic application of moxifloxacin in sheep against variety of bacterial infections.

Key words: Dosage regimen, Intravenous, Moxifloxacin, Pharmacokinetics, Sheep.

Fluoroquinolones are considered to have a concentration-dependent effect, although a time-dependent bactericidal effect against some Gram-positive bacteria has also been described (Dalhoff *et al*.,1996). They also have characteristics like a wide spectrum of bactericidal activity, a large volume of distribution, low plasma protein binding and a relatively low minimum inhibitory concentration (MIC) against susceptible target microorganisms (Brown, 1996). Moxifloxacin, fourth generation fluoroquinolone is one of the broadly used among the fluoroquinolones and has gained an imperative use in veterinary medicine as in human medicine. It is active against gram-positive, gram-negative, and aerobic facultative bacteria. There have been some limited data published on moxifloxacin pharmacokinetics in animals. For example, horses (Gardner *et al*., 2004), rabbits (Cárceles *et al*., 2006 and Fernandez-Varon *et al*., 2005), goats (Cárceles *et al*., 2007; Fernandez-Varon *et al*., 2006 and Patel *et al*., 2009[a]). Sheep (Goudah, 2008 and Cárceles *et al*., 2009), calves (Goudah and Hasabelnaby 2010 and Patel *et al*.,2009[b]) and camels (Abd el-atya *et al*., 2007). In view of the large differences in disposition kinetic behavior of drugs due to species variation, age, assay methods and pharmacokinetic evaluation with compartmental and non-compartmental models, this study was planned to investigate the plasma pharmacokinetics characteristics and dosage regimen of moxifloxacin in sheep after single dose intravenous administration.

MATERIALS AND METHODS

Experimental animals

Six apparently healthy Patanwadi female sheep of body weight ranging from 25 to 30 kilogram were selected for the study. Sheep were housed at the livestock research station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. During this period, they were subjected to clinical examination in order to exclude the possibility of any disease. Any biological or

pharmaceutical agents were not administered prior to or at the time duration of study. The animals were maintained on an antibiotic free standard diet and ad libitum water was provided throughout the experiment period. The experimental protocol was approved by Institutional Animal Ethics Committee and all the measures for welfare of experiment animal were taken as per Committee for Purpose of Control and Supervision on Experiment on Animal guide line.

Drug and Chemicals: A single dose of moxifloxacin injection (100 $mg.ml^{-1}$ formulation, Intas pharmaceutical Pvt. Ltd. Gujarat, India) was used in the study. Water, acetonitrile and tetrabutyl ammonium hydrogen sulfate of HPLC grade were procured from S. D. Fine Chem. Ltd, Mumbai. 0.067M disodium hydrogen phosphate and hydrochloric acid of analytical grade were purchased from S. D. Fine Chem. Ltd, Mumbai.

Experimental design and drug administration: A single dose of moxifloxacin injection was injected at dose rate of 5.0 $mg.kg^{-1}$ body weight through intravenous route via jugular vein in each sheep. Blood samples (approximately 5.0 ml) were collected from each treated sheep in clean sterilized centrifuge tube with appropriate amount of heparin with the help of an intravenous catheter (Venflon) fixed into jugular vein at 0 time (before drug administration) and at 0.083, 0.167, 0.5, 1, 2, 4, 8, 12 and 24 h after i.v drug administration in order to confirm persistence of drug in blood. Plasma was separated after centrifugation of blood samples at 1600 revolutions per minute (rpm) for 10 min. The plasma samples were transferred to cryo-vials (3 ml capacity) and stored at -20°C until analyzed. Plasma concentrations of moxifloxacin were measured using a modified HPLC method (Siefert *et al*., 1999).

Moxifloxacin HPLC Method: The apparatus utilized for drug assay was the Agilent 1100 series. It consisted of modal LC-9A (gradient solvent delivery pump), a modal RF-551 Fluorescence Detector and modal SIL-6B automatic sampler. Chromatographic separation was done using Supelco C18 (250 mm X 4.6 mm 5µ) column at room temperature.

The mobile phase consisted of a mixture of buffer and acetonitrile (80:20). The buffer was prepared by dissolving 10 gm of tetrabutyl ammonium hydrogen sulphate in one liter of deionised water and the diluent was prepared by 0.067M disodium hydrogen phosphate in water, adjusted to pH 7.5. The buffer and acetonitrile were separately sucked gradiently into HPLC system, mixed in 3:1 ratio and pumped in to the column at a flow rate of 1.0 $ml.min^{-1}$ at $20^{o}C$ temperature and the excitation was monitored at wavelength of 296 nm and emission was monitored at wavelength of 504 nm.

Plasma proteins were precipitated by addition of 2 ml each of plasma and acetonitrile in a centrifuge tube and shaken on a vortex mixture for 10 seconds. This was followed by centrifugation for 10 min at 5000 rpm. Thereafter 3.0 ml of diluent was added to 2.0 ml of clear supernatant fluid in a glass tube and mixture was vortexed for 10 seconds. This was transferred into inserts (automatic sampler vial) from which 50µl was injected into HPLC system.

Pharmacokinetic analysis: Various pharmacokinetic parameters like absorption, distribution, elimination half-life, apparent volume of distribution and total body clearance were calculated by PK Solutions Version 2.0 computer software, USA. This program uses non-compartmental model of pharmacokinetic analysis of long acting moxifloxacin.

For bactericidal antimicrobial drugs, such as moxifloxacin, the priming and maintenance approach to dosage is desirable. The maintenance dose (D') of moxifloxacin based on the desirable minimal therapeutic concentration of 0.25 $\mu g.ml^{-1}$ in plasma at time intervals of 12h is calculated using following equation:

$$D' = CP^{-\infty}(min) \bullet V_d \bullet (e^{\beta\tau} - 1)$$

Where $CP^{-\infty}$ is the minimum inhibitory concentration, Vd is the apparent volume of distribution, e represents the base of natural logarithm, β is overall elimination rate constant and τ (Tau) is the dosage interval

(Baggot, 1977). The priming dose was obtained by omitting -1 from the right side of the above equation.

RESULTS

The semi logarithmic plot of moxifloxacin concentrations in plasma versus time following single dose intravenous administrations given at the dose rate of 5.0 mg.kg^{-1} body weight in female sheep are given in Figure 1. The plasma level of moxifloxacin following i.v. at 0.083 (5 min) was recorded as 10.249±0.809 µg.ml^{-1}. Plasma levels rapidly declined to 5.611±0.075 µg.ml^{-1} at 0.167 h (15 min). The drug was detected up to 24 h after i.v. administration. The minimum inhibitory contraction of moxifloxacin is 0.1- 0.5 µg.ml^{-1} (Woodcock *et al*., 1997). Table 1 depicts the dosage regimen of moxifloxacin in sheep for i.v. route of administration to maintain the minimum therapeutic concentration 0.25 µg.ml^{-1} in plasma.

Table1: Pharmacokinetic data of moxifloxacin after single intravenous administration (5.0 mg/kg body weight) in sheep (n=6).

Pharmacokinetic Variables	Unit	Mean ± S.E.
C_p^O	µg.ml^{-1}	7.378±0.318
$K_{(a)}$	h^{-1}	1.070±0.065
B	h^{-1}	0.181±0.011
$t_{½ K(a)}$	h	0.660±0.041
$t_{½\beta}$	h	3.908±0.258
C_{max}	µg.ml^{-1}	10.249±0.809
AUC	µg.h.ml^{-1}	11.254±0.189
AUMC	µg.h^2.ml^{-1}	31.685±1.553
$Vd_{(area)}$	L.kg^{-1}	2.512±0.179
$Vd_{(ss)}$	L.kg^{-1}	1.252±0.058
Cl_B	L.h.kg^{-1}	0.445±0.007
MRT	h	2.814±0.126
K_{12}	h^{-1}	0.285±0.030
K_{21}	h^{-1}	0.287±0.025
K_{10}	h^{-1}	0.678±0.034

$K_{(a)}$:distribution rate constant; β:elimination rate constant; $t_{½K(a)}$: half-life of distribution phases; $t_{1/2\beta}$: elimination half life; Cmax: maximum drug concentration; AUC: total area under plasma drug concentration-time curve; AUMC: area under first of moment curve; Vd(area): volume of distribution based on area; Vd(ss): volume of distribution at steady state; Cl_B : total body clearance; MRT : mean residence time; K_{12} : rate constant of a drug from central to peripheral compartment; K_{21}: rate constant of a drug from peripheral to central compartment; K_{10} / K_{el} : elimination rate constant from central compartment

Table 2: Priming and maintenance doses of moxifloxacin in sheep

MIC (µg.ml^{-1})	Dosage interval (h)	Priming dose (mg.kg^{-1})	Maintenance dose (mg.kg^{-1})
0.25	12	5.5	5.0

The detailed pharmacokinetic parameters of long acting moxifloxacin calculated for sheep were presented in the table 2. The values of distribution rate constant (α) varied from 0.848 to 1.267 h^{-1} with a mean of 1.070±0.065 h^{-1}. The distribution half-life ($t_{1/2\alpha}$) ranged between 0.560 to 0.818 h. with a mean of 0.660 ± 0.041 h. The elimination

rate constant (β) varied from 0.140 to 0.221 h^{-1} with a mean of 0.181 ± 0.011 h^{-1}. The elimination half-life ($t_{1/2}$) ranged between 3.136 to 4.953 h. with a mean of 3.908 ± 0.258 h.

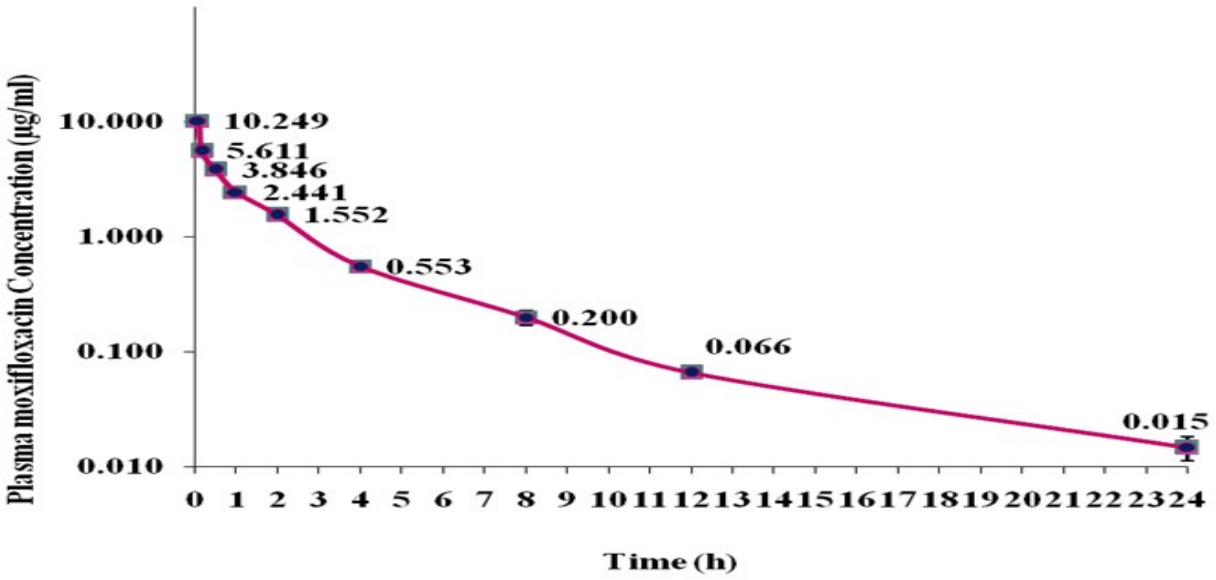

Figure 1: Semilogarithmic plot of moxifloxacin concentration in plasma versus time after single intravenous administration given at the dose rate of 5.0 $mg.kg^{-1}$ body weight in sheep (n=6).

The average rates of transfer of drug from central to the tissue compartment (K_{12}), tissue to the central compartment (K_{21}) and elimination from the central compartment (K_{el} or K_{10}) were calculated to be 0.285±0.030, 0.287±0.025 and 0.678±0.034 h^{-1}, respectively.

DISCUSSION

Following single dose intravenous administration of moxifloxacin the distribution of the drug was relatively higher in sheep ($t_{½K(\alpha)}$ = 0.660±0.041h) as compared to goats (0.36±0.14 h) (Fernandez-Varon *et al*., 2006) and lactating ewe (0.22±0.02 h) (Goudah, 2008) in the present study. The $t_{½\beta}$ (3.908±0.258 h) of moxifloxacin in the present study was longer than as compared to calves (3.29 h) (Goudah and Hasabelnaby 2010), lactating ewes (1.77±0.23 h) (Goudah, 2008), lactating goats (1.31±0.64 h) (Cárceles *et al*., 2007), camel (1.87±0.16 h) (Abd el-atya *et al*., 2007) and rabbit (1.84±0.12 h) (Fernandez-Varon *et al*., 2005). However, it was also found comparatively lower than goats (4.121±0.302 h) (Patel *et al*., 2009[a]) and buffalo calves (4.121±0.302 h) (Patel *et al*., 2009[b]) following intravenous administration of moxifloxacin.

Volume of distribution at steady state ($Vd_{(ss)}$) of drug observed in sheep (1.252±0.058 $L.kg^{-1}$) was in agreement with lactating goats (0.79±0.08 $L.kg^{-1}$) (Fernandez-Varon *et al*., 2006), lactating ewes (0.84±0.12 $L.kg^{-1}$) (Goudah, 2008) and buffalo calves (0.26 $L.kg^{-1}$) (Patel *et al*., 2009[b]). Similarly high value of $V_{d(ss)}$ (5.0, 2.08, 1.95 $L.kg^{-1}$) were also reported in goats (Patel *et al*., 2009[a]), rabbits (Cárceles *et al*., 2006 and Fernandez-Varon *et al*., 2005) respectively. The good tissue distribution may be related to low molecular weight of the drug's and high affinity for lipid-bearing tissues.

In the present study, mean value of Cl_B (0.445±0.007 $L.h.kg^{-1}$) is in proximity to the values of Cl_B reported in goats (0.43±0.02 $L.h.kg^{-1}$) (Fernandez-Varon *et al*., 2006), sheep (0.39±0.04 $L.h.kg^{-1}$) (Cárceles *et al*., 2009) and in ewes (0.34±0.04 $L.h.kg^{-1}$) (Goudah, 2008).

The AUC of moxifloxacin (11.254±0.189 $\mu g.h.ml^{-1}$) was markedly lower than the AUC value reported in ewes (14.74±2.16 $\mu g.h.ml^{-1}$) (Goudah, 2008) and male camels (14.72±0.69 $\mu g.h.ml^{-1}$) (Abd el-atya *et al*.,

2007). The AUC observed in sheep was in agreement with the values of 11.71±0.67 $mg.h.l^{-1}$ in goats (Fernandez-Varon *et al*., 2006). However lower values of AUC (6.28±0.13 $\mu g.h.ml^{-1}$) has also been reported in rabbits (Fernandez-Varon *et al*., 2005) following moxifloxacin in rabbits. The difference in AUC values may be due to species variation and difference in formulation.

The ultimate objective of present disposition study was to compute dosage regimen of moxifloxacin in sheep for i.v. route for the treatment of infectious diseases caused by susceptible bacteria. For maintaining MIC 0.25 $\mu g.ml^{-1}$ in plasma, moxifloxacin should be given in the dose of 5.5 $mg.kg^{-1}$ body weight followed by 5.0 $mg.kg^{-1}$ body weight at 12 h intervals by i.v. route.

CONCLUSION

After intravenous administration at the dose rate of 5.0 $mg.kg^{-1}$ the effective therapeutic concentration of moxifloxacin was maintained up to 24h. So, for clinicians this would be very economical and expedient in treating infectious diseases caused by bacteria in sheep.

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