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PHARMACOKINETICS AND DOSAGE REGIMEN OF CIPROFLOXACIN FOLLOWING SINGLE INTRAMUSCULAR ADMINISTRATION IN NILI/RAVI BUFFALOS

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ABSTRACT

Ciprofloxacin is second generation fluoroquinolone antibiotic which is being used to treat various infectious diseases of man and animals. Due to wide-spread use of this antimicrobial in veterinary clinics, but little information regarding its disposition, this project was carried out to determine the PK and optimal dosage regimen of ciprofloxacin in *Nili/Ravi* buffalos. Eight healthy adult buffalos of *Nili/Ravi* breed (average ± SE weight of 368 ± 44 kg) were maintained under same conditions of environment and management. A dose of 5 mg/Kg was administered in the neck muscles of each animal through injection. After injecting drug, samples of blood were taken at various times and kept in centrifuge tubes having heparin. In every animal, a control sample of blood was drawn before injecting drug. HPLC was used to determine drug concentration in the samples. The value for half life elimination (t1/2 ß) was 3.05 ± 0.20 hours. Mean ± SE value for volume of distribution (Vd) was 1.09 ± 0.06 L/kg, for AUC was 20.28 ± 1.13 µg.hr/ml and for total body clearance (CL) was 0.25 ± 0.02 L/hr/kg. An optimal dosage regimen for intramuscular administration of ciprofloxacin in *Nili/Ravi* buffalos was calculated using these parameters which was 17.86 mg/kg, recommended to be given after every 24 hours. We came to conclusion that our calculated dose in local buffalos was significantly higher than the recommended dose of manufacturer and to avoid antimicrobial resistance, this locally investigated dosage regimen should be strictly followed in local buffalos.
Keywords: Ciprofloxacin, Buffalos, Dosage regimen, Pharmacokinetics, HPLC

INTRODUCTION

Since their introduction, the fluoroquinolones (FQs) have been the focus of attention as synthetic antimicrobial agents. They are extensively used in veterinary health programs due to their good bioavailability and pharmacokinetic (PK) characteristics (Vancutsem *et al.,* 1990; Papich and Riviere, 2009). FQs produce their effect through concentration dependent killing mechanism, which means the optimal effect is produced by administration of high doses over a short period of time (Drusano *et al.,* 1993). This type of killing by FQs is also associated with a prolonged post antibiotic effect (Aliabadi and Lees, 2001). All FQs are bactericidal and target DNA gyrase (in gram-negative bacteria) and topoisomerase IV (in gram positive bacteria) (Blondeau *et al.,* 2004). A steady-state intrabacterial concentration of FQs is obtained in very short time due to their very rapid accumulation inside the bacteria (Smith, 1986).

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Ciprofloxacin is second generation FQ which is being used to treat various infectious diseases of man and animals (Stein, 1996). In some countries like USA, its application in food animals is barred but in other countries like Pakistan it extensively used in food producing animals. A review of the relevant literature shows that PK of ciprofloxacin has not been studied in local animals of human interest. Due to wide-spread use of this antimicrobial in veterinary clinics, but little information regarding its disposition, this project was carried out to determine the PK and optimal dosage regimen of ciprofloxacin in *Nili/Ravi* buffalos. It is hoped that the study will help in optimizing the dosage of ciprofloxacin in local buffalos.

MATERIALS AND METHODS

Experimental Animals and Drug Administration

The study was conducted at the experimental farms of the Department of Livestock Management, University of Agriculture,

Faisalabad, Pakistan during the month of January, 2007. Eight healthy adult buffalos of *Nili/Ravi* breed (average ± SE weight of 368 ± 44 kg) were maintained under same conditions of environment and management. Animals had free access to fodder and water. An injectable preparation of ciprofloxacin was administered in the neck muscles of each animal through injection @ 5 mg/Kg.

Collection of Blood Samples

After injecting drug, samples of blood were taken at various times (0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 10 hours) and kept in centrifuge tubes having heparin. In every animal, a control sample of blood was drawn before injecting drug. The pH of fresh blood samples was recorded by a pH meter (Beckman HS, Germany) with a glass electrode at 37°C. Blood samples were centrifuged and plasma was separated and stored at - 20°C until analysis. The concentration of ciprofloxacin in plasma was determined by using high performance liquid chromatograph (HPLC). The method has already been reported in detail in our previous publication (Iqbal et al., 2011).

Pharmacokinetic Analysis

The plasma concentration versus time profile of ciprofloxacin after intramuscular administration in each animal was used to establish various pharmacokinetic parameters. A standard two-stage (STS) approach was used in which each animal's data was analyzed separately, then averaged to produce the mean value for the group. A two-compartment open model was fitted to the data with a computer programme, MW/PHARM version 3.02, a MEDIWARE product, Holland (Netherlands). Least Square Regression Analysis was applied to discriminate the best model and correlation coefficient was taken as measure of goodness-of-fit.

Dosage Regimen

Based on pharmacokinetic/pharmacodynamic (PK/PD) parameters; optimal dosage regimen of ciprofloxacin to be repeated after 24 hours interval was calculated in adult *Nili/Ravi* buffalos using the following equation:

$$\text{Dose} = \frac{\text{CL} \cdot (\text{AUC/MIC}) \cdot \text{MIC}}{\text{fu} \cdot \text{F} \cdot 24\ \text{hr}}$$

To derive a dose for this study, we used the average clearance value produced by our study (reported as CL/F), an estimated protein fraction unbound from other studies (fu), and MIC values across a range for the pathogens obtained from these animals. The AUC calculated from a compartmental approach is from time zero to infinity. This AUC is used for dose calculations because it is recognized that, at steady-state, AUC_{24} from a dose administered once per day (every 24 hours), the value of 24 in the above equation cancels out.

RESULTS

Pharmacokinetic Analysis

The mean ± SE values for plasma concentration of ciprofloxacin after intramuscular injection in eight *Nili/Ravi* buffalos have been plotted on a semilogarithmic scale against time after injection in Fig 1.

Pharmacokinetic parameters (mean ± SE) of ciprofloxacin in buffalos are presented in Table 1. The time to reach maximum concentration (Tmax) was observed as 0.87 ± 0.03 hours. The values of Cmax, A and B were 4.89 ± 0.28, 7.22 ± 0.91 and 3.56 ± 0.30 µg/ml, respectively. The mean ± SE values for half life of absorption (t1/2 abs), distribution (t1/2 α) and elimination (t1/2 ß) were 0.45 ± 0.03, 0.45 ± 0.03 and 3.05 ± 0.20 hours, respectively. Mean ± SE value for volume of distribution (Vd) was 1.09 ± 0.06 L/kg, for AUC was 20.28 ± 1.13 µg.hr/ml and for total body clearance (CL) was 0.25 ± 0.02 L/hr/kg.

Dosage Regimen

Based on the pharmacokinetic / pharmacodynamic parameters, optimal dosage regimen of ciprofloxacin was calculated in adult *Nili/Ravi* buffalos. The calculations of dose were based on the minimum inhibitory concentration (MIC) of ciprofloxacin in blood, fraction of the drug not bound to plasma proteins (fu) which was taken as 0.70 and ratio of AUC/MIC which was taken as 100. The value of 100 is used in this equation because it represents a conservative consensus as reported in review of the relevant literature (McKellar *et al.*, 2004). Occasionally, higher values (e.g., AUC/MIC) of 125 have been cited when protein binding was not factored into the equation. McKellar's review also points out that for some organisms (e.g., gram-positive) lower ratios might be possible, but for this

study our desire was to predict a single dose that would encompass both gram-positive and gram-negative bacteria. The MICs of ciprofloxacin 0.02, 0.03, 0.1, 0.2, 0.3, 0.4, 0.5 and 1.0 µg/ml were used in the calculations because they represent the range of values for pathogens that cause infections in buffalos. The intramuscular dose of ciprofloxacin in mg/kg body weight for 24 hour dosing interval in adult *Nili/Ravi* buffalos is presented in Table 2. In *Nili/Ravi* buffalos**,** at MIC 0.5 µg/ml, the calculated intramuscular dose is 17.86 mg/kg, to be repeated after 24 hour interval.

Table – 1: Mean ± SE values for the disposition kinetics of ciprofloxacin following intramuscular administration of 5 mg/kg body weight in each of the 8 adult *Nili/Ravi* buffalos.

Parameters	Units	Buffalos (n = 8)
C_{max}	(µg/ml)	4.89±0.28
t_{max}	(hr)	0.87±0.03
K_{abs}	(hr^{-1})	1.57±0.08
$t_{1/2abs}$	(hr)	0.45±0.03
A	(µg/ml)	7.22±0.91
α	(hr^{-1})	1.57±0.08
$t_{1/2}\alpha$	(hr)	0.45±0.03
B	(µg/ml)	3.56±0.30
β	(hr^{-1})	0.23±0.01
$t_{1/2}\beta$	(hr)	3.05±0.20
V_c	(L/kg)	0.49±0.04
Vd	(L/kg)	1.09±0.06
K_{el}	(hr^{-1})	0.54±0.04
k_{12}	(hr^{-1})	0.55±0.04
k_{21}	(hr^{-1})	0.71±0.08
CL	(L/hr/kg)	0.25±0.02
AUC	(µg.hr/ml)	20.28±1.13

A and *B*, *Y*-axis intercept terms; C_{max}, peak plasma concentration of drug; t_{max}, time to reach peak plasma concentration; K_{abs}, absorption rate constant; α, distribution rate constant; K_{el}, elimination rate constant; β, overall elimination rate constant; $t_{1/2abs}$, absorption half-life; $t_{1/2}\alpha$, distribution half-life; $t_{1/2}\beta$, elimination half-life; k_{12}, rate constant from central to peripheral compartment; k_{21}, rate constant from peripheral to central compartment; *Vc*, volume of distribution in the central compartment; *Vd*, volume of distribution ; *AUC*, area under the concentration - versus time curve from time zero to infinity; *CL*, total body clearance.

Table – 2: Intramuscular dosage regimens of ciprofloxacin (mg/kg) to be repeated after 24 hours interval in adult *Nili/Ravi* buffalos in order to produce AUC/MIC value > 100 for the unbound drug (fu).

MIC (µg/ml)	Dose (mg/kg)
0.02	0.71
0.03	1.07
0.05	1.79
0.10	3.57
0.20	7.14
0.30	10.71
0.40	14.28
0.50	17.86
1.00	35.71

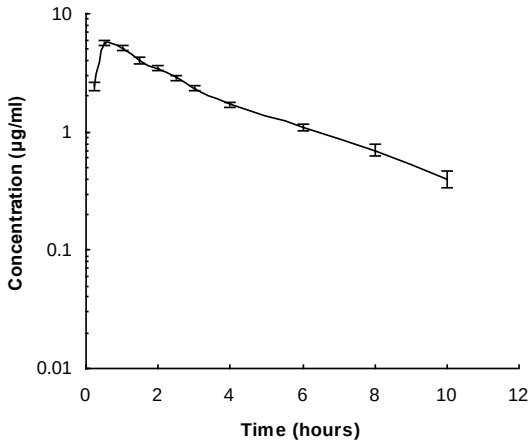

Fig – 1: Mean ± SE plasma concentration of ciprofloxacin on a semilogarithmic scale versus time after single intramuscular administration (5 mg/kg) in 8 *Nili/Ravi* buffalos.

DISCUSSION

Pharmacokinetic Analysis

Following intramuscular administration of ciprofloxacin in buffalos, the absorption was rapid as mean absorption half life was 0.45 hours. The distribution half life of ciprofloxacin in buffalos, 0.45 hours, was shorter than 0.89 hours (Saini and Srivastava, 2001) but longer than 0.28 hours in buffalo calves following intravenous administration of enrofloxacin at the rate of 4 mg/kg body weight (Kumar *et al.*, 2003).

The value of elimination half life, 3.05 hours, of ciprofloxacin in present study, was longer than 1.97 hours after its intramuscular administration in buffalo bulls (Verma *et al.*, 1999) and 2.92 hours (Kumar *et al.*, 2003) and 2.90 hours (Sharma *et al.*, 2003) after intravenous administration of enrofloxacin in buffalo calves. The value (3.05 hours) was shorter than 3.88 hours for ciprofloxacin (Saini and Srivastava, 2001), 4.99 hours for danofloxacin (Sappal *et al.*, 2006) following intravenous administration and 7.45 hours for gatifloxacin (Raipuria *et al.*, 2006) after intramuscular administration to buffalo calves. These studies indicate rapid distribution of fluoroquinolones reflected through the slower elimination. It is evident from the present study that the elimination half life of ciprofloxacin in local buffalos (3.05 hours) was shorter than most of the literature values. It has been appreciated that half life is a derived parameter which changes as a function of both clearance and volume of distribution (Gibaldi, 1984) so, shorter half life may be attributed to the lower value of Vd in the buffalos of present study than the most reported values in the literature.

The apparent volume of distribution (1.09 L/kg) in present study was higher than 0.61 L/kg for enrofloxacin in buffalo bulls (Verma *et al.*, 1999). However, it was lower than 3.97 L/kg for ciprofloxacin (Saini and Srivastava, 2001), 5.33 L/kg and 6.90 L/kg for enrofloxacin (Kumar *et al.*, 2003; Sharma *et al.*, 2003), 4.11 L/kg for danofloxacin (Sappal *et al.*, 2006) and 3.2 L/kg for gatifloxacin (Raipuria *et al.*, 2006) in buffalo calves. Further, higher values have been reported in cows (2.5 L/kg) (Nouws *et al.*, 1988), sheep (1.90 L/kg) (Munoz *et al.*, 1996) and chickens (2.02 L/kg) (Atta and Sharif, 1997). Besides intra and inter species biological variations, a possible explanation for the lower value of Vd in present study may be linked to the higher extrapolated zero time drug concentration (B) as compared to its lower values in above cited studies. It was investigated in a study that in sheep, a 29.6 % increase in B was primarily responsible for 24.5 % lower Vd of sulphadimidine in summer than that in winter (Nawaz and Nawaz, 1983). Another study showed that in alloxan diabetic dogs, osmotic diuresis lead to water loss or dehydration accompanied with higher value of B and decrease in Vd when compared with normal dogs (Nawaz *et al.*, 1982).

Total body clearance in local buffalos (0.25 L/hr/kg) is comparable to 0.21 L/hr/kg for enrofloxacin in buffalo bulls (Verma *et al.*, 1999) but lower than 0.71 L/hr/kg for ciprofloxacin (Saini and Srivastava, 2001), 1.94 L/hr/kg and 1.67 L/hr/kg for enrofloxacin (Kumar *et al.*, 2003; Sharma *et al.*, 2003), 0.30 L/hr/kg for gatifloxacin (Raipuria *et al.*, 2006) and 0.67 L/hr/kg for danofloxacin (Sappal *et al.*, 2006) in buffalo calves. A lower total body clearance (CL) in local buffalos than that reported in foreign counterparts may be related to the lower value of Vd (1.09 l/kg) in present study as compared to higher reported values.

Although an intravenous dose was not administered, which would have defined more precisely the pharmacokinetic parameters, these results show that ciprofloxacin in these buffalos has the general pharmacokinetic characteristics of a typical fluoroquinolone antimicrobial agent. Also, because the values of Vd/F and CL/F determined from this study are in general agreement with the value obtained from intravenous studies, it may indicate high bioavailability and a value of F that is near 100 % from the intramuscular injection used in this study. However, this cannot be confirmed without a bioavailability study.

Dosage Regimen

Inadequate antibiotic concentrations can be one of the causes of ineffectiveness (Kiss *et al.*, 1976; Nicolau, 2003). Suboptimal concentrations may also contribute to antimicrobial resistance. Also, Peter Lees stated that it was exposure, and especially exposure to sub-optimal drug concentrations that was the most important single factor in resistance emergence and its subsequent spread (Lees *et al.*, 2008). The susceptibility of bacteria to the action of ciprofloxacin varies not only between species but also between strains within the same species resulting in a very wide range of minimum inhibitory concentration (MIC) against the susceptible microbes. For the majority of the organisms that are susceptible to enrofloxacin and ciprofloxacin, the reported MIC values are 0.1 - 1 µg/ml (Bauditz, 1990) and even < 0.5 µg/ml (Giguere *et al.*, 1996). Lower range of MIC of ciprofloxacin for bacteria isolated from buffalo calves was 0.10 µg/ml (Saini and Srivastava, 2001) and ≥ 0.1 µg/ml (Kaartinen *et al.*, 1997). MIC value of enrofloxacin for susceptible bovine pathogens

has also been reported to be 0.06 µg/ml (Davis *et al.,* 2007). The information provided on enrofloxacin's FDA-approved label (Baytril 100) in the United States lists the MIC_{90} for susceptible pathogens to be 0.03 to 0.06 µg/ml for non-Mycoplasma organisms. An MIC_{90} for ciprofloxacin was reported as 0.015 - 0.06 µg/ml against gram negative while 0.25 - 0.5 µg/ml against most of the gram positive veterinary pathogens and it was also reported that ciprofloxacin MIC values were similar to MIC values of enrofloxacin for *P. multocida* and *H. somnus* and slightly lower for *M. haemolytica* (Prescott and Yielding, 1990). Based on these observations, a range of MIC values of, 0.02 - 1.0 µg/ml was used to develop a *target attainment table* (Table 2) to provide estimated doses needed to attain an AUC/MIC target of 100, for unbound drug. This may be used to determine the optimal dosage regimen suggested in *Nili/Ravi* buffalos. Based on these calculations, we suggest an optimal dosage regimen of ciprofloxacin in local buffalos as 17.86 mg/kg to be repeated intramuscularly after 24 hours and will become 18 mg/kg in the field conditions. The dose recommended by the manufacturer of the pharmaceutical preparation of ciprofloxacin (5 mg/kg/24 hours) attained a target of AUC/MIC of 100 for unbound drug (fu) only for bacteria with MIC < 0.2 µg/ml (Table 2). Other investigators have recommended higher doses or more frequent intervals. An intravenous dose of 10 mg/kg ciprofloxacin was suggested to be repeated after 12 hours in goats (Garcia Ovando *et al.,* 2000) while intramuscular dose of 5 mg/kg body weight was suggested after every 12 hours in cross-bred cow calves (Singh and Srivastava, 2000). In another study, a dose of 3 mg ciprofloxacin/kg body weight was recommended to be repeated after 12 hours interval in buffalo calves (Saini and Srivastava, 2001).

CONCLUSIONS

Based on the findings of the present study it was concluded that the dosage regimen of ciprofloxacin in local buffalos recommended by the manufacturers was much lower than the suggested dosage regimen investigated in the present study.

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