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Pharmacokinetics of Enrofloxacin after Single Intravenous and Oral Bolus Administration in Broiler Chicken

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Abstract The pharmacokinetics and bioavailability of enrofloxacin was compared in Cobb strain broiler chicken after intravenous and oral administration of enrofloxacin at the rate of 10mg.kg^{-1}. The concentration of enrofloxacin at various time intervals in plasma was determined by HPLC and the pharmacokinetic parameters were calculated by non compartmental approach. AUC was significantly high in i.v. route (32.72±1.15 *vs* 25.35±1.92μg.h.mL^{-1}) whereas highly significant increase in MRT (15.81±0.54 *vs* 8.86±0.23h), $V_{d\ area}$ (4.69±0.16 *vs* 3.04±0.09 L.kg^{-1}) and $t_{1/2\beta}$ (10.57±0.35 *vs* 6.84±0.15h) were noticed in oral route when compared to i.v. route. The C_{max} of 1.63±0.12μg.mL^{-1} was attained at t_{max} of 3.58±0.61h and absolute bioavailability was 77.47±5.86% after oral administration. PK/PD integration revealed that the dose (10mg.kg^{-1}) was capable of treating only moderately sensitive organisms with MIC ≤0.125μg.mL^{-1} and increase in dosage is needed for less sensitive organisms.

Keywords *Bioavailability; Broiler Chicken; Enrofloxacin; Pharmacokinetics; PK/PD Integration*

1. Introduction

Enrofloxacin, a fluoroquinolone antibacterial developed exclusively for veterinary use is still one of the highly used antibacterial in poultry to treat outbreaks of various bacterial diseases especially chronic respiratory disease, mycoplasmosis *etc.* Overuse of enrofloxacin has resulted in development of resistant bacterial populations and may also reduce the clinical efficacy (Sumano and Gutierrez, 2001). Though many pharmacokinetic studies were reported for enrofloxacin, the present study was

conducted in order to assess whether the dosage regimen followed is sufficient to obtain clinical cure in Cobb strain of broiler chicken reared in and around Namakkal region of Tamil Nadu, India, since wide variation in pharmacokinetic parameters were noticed between various studies.

2. Materials and Methods

2.1. Experimental Birds

The study was conducted in 24 commercial six weeks old apparently healthy Cobb broiler chicken of either sex weighing 2 to 2.5kg. The birds were purchased from commercial poultry farm at the age of four weeks and acclimatized for two weeks period. The birds were reared in individual cages under standard and uniform conditions with natural day-night cycle and fed *ad libitum* feed and water free of antibacterial. The experimental trial on birds was approved by Institutional Animal Ethics Committee, Veterinary College and Research Institute, Namakkal-2.

2.2. Drug Administration

The birds were divided into two groups of 12 each and enrofloxacin (M/s. Himedia, India) was administered at the rate of 10mg.kg^{-1} body weight through i.v. and oral route. The drug was dissolved in 0.1N NaOH to prepare 1.5 per cent solution and further diluted in normal saline and drinking water for i.v. and oral administration, respectively. The method of drug administration and collection of blood samples are mentioned in Table 1.

Table 1: *Experimental Design*

Group	Route	Method	Timing of Blood Collection
I	IV	Medial Metatarsal vein	0, 0.08, 0.167, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0, 36.0 and 48.0h
II	Oral	Intra crop with semi rigid plastic tube	0, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0, 36.0 and 48.0h

Blood sample was collected in heparinised vials and plasma was separated by centrifuging at 950g for 15 min and stored at -80°C until analysis.

2.3. HPLC Analysis

Plasma samples were assayed for enrofloxacin concentration by reverse phase high performance liquid chromatography. The HPLC system (Schimadzu, Japan) consisted of an isocratic pump (LC-10AT double pump), a rheodyne manual injector with 20µL loop, C_{18} column (5µ particle size, 4.6 x 250mm length, Lichrosphere, Merck), Column oven (CTO- 10 AS vp) maintained at 40°C, Photodiode array UV-Vis detector and LC solution chromatopak software.

The plasma samples were extracted as per the method of (Nielsen and Hansen, 1997) and quantified as per (Kung *et al.*, 1993). The isocratic mobile phase consisted of acetonitrile: methanol: water (17:3:80, v/v/v) with 0.4% triethylamine, 0.4% orthophosphoric acid (85%, v/v) and pH adjusted to 3.0 with triethylamine. The scan range was 220 to 400nm and the detection wavelength was 278nm. The flow rate of mobile phase was 1.0mL.min^{-1} and run time was 10min.

The concentration of enrofloxacin in chicken plasma was obtained from the calibration curve. Standard curve was prepared for concentrations ranging from 0.01 to 10µg.mL^{-1} by spiking enrofloxacin in drug free chicken plasma and the linearity of the method was examined by linear

regression analysis of the standard curve. The recovery of enrofloxacin was 97.11 per cent and coefficient of variation (CV) was 4.22 per cent. Limit of detection and quantification were 0.01 and 0.025μg.mL^{-1}, respectively. The method was found to be linear and reproducible in the concentration range of 0.025 to 10μg.mL^{-1} for enrofloxacin. The intra- and inter-day assay CV was 3.59 and 4.08 per cent, respectively.

2.4. Pharmacokinetic Analysis

Pharmacokinetic parameters were calculated from the concentration of enrofloxacin detected in plasma by non compartmental analysis based on statistical moments theory (Singh, 1999) by using PK functions software.

2.5. Statistical Analysis

The plasma concentration and pharmacokinetic parameters are expressed as mean±SE and between groups comparison was made by students paired t test (Snedecor and Cochran, 1994).

3. Results and Discussion

The mean (±SE) plasma concentration *vs* time data of enrofloxacin after single i.v. and oral bolus dose is presented in Table 2. The mean plasma concentration was 6.01 ±0.26μg.mL^{-1} at 0.08h after i.v. administration and the concentration decreased gradually to 1.74±0.15μg.mL^{-1} at 6h until which it differed significantly from the concentration obtained from oral route. There after no significant difference in plasma concentration was noticed between i.v. and oral route and concentration was detected up to 36 and 48h in i.v. and oral route, respectively. In oral route plasma concentration was detected from 0.25h (0.36±0.04μg.mL^{-1}) which increased gradually and reached maximum plasma concentration of 1.48±0.10μg.mL^{-1} at 4h after which it declined to 0.09±0.01μg.mL^{-1} at 48h. Further, mean concentration exceeding 0.5μg.mL^{-1} was present up to 12h in both the routes.

Table 2: *Comparison of Mean Plasma Concentrations (μG.Ml^{-1}) after Single IV and Oral Bolus Dose of Enrofloxacin in Broiler Chicken*

Time (h)	Mean±SE	
	IV	Oral
0.08	6.01 ±0.26	-
0.167	5.64±0.28	-
0.25	5.16**±0.26	0.36±0.04
0.5	4.74**±0.24	0.56±0.02
1	3.96**±0.20	0.87± 0.04
1.5	3.63**±0.20	1.19±0.07
2	3.17**±0.14	1.46±0.11
4	2.54**±0.14	1.48±0.10
6	1.74*±0.15	1.33±0.09
8	1.32^{NS}±0.12	1.11^{NS}±0.07
10	0.84^{NS}±0.06	0.90^{NS}±0.07
12	0.54^{NS}±0.04	0.66^{NS}±0.06
24	0.29^{NS}±0.02	0.34^{NS}±0.04
36	0.10^{NS}±0.01	0.16^{NS}±0.03
48	ND	0.09±0.01

– Sample not collected
ND- Not detected
* Significant (p<0.05)
** Significant (p<0.01)

(Anadon *et al.*, 1995) reported higher concentration of 24.06±0.43μg.mL^{-1} at 10min whereas (Jakubowski *et al.*, 2010) reported slightly lower concentration of 4.17μg.mL^{-1} at 5min after i.v administration. Concentration exceeding 0.5μg.mL^{-1} persisted for about 12h in both the studies which was similar to the present study whereas it was up to 24h in the study conducted by (Silva *et al.,* 2006). These differences might be due to the difference in the strain and age of bird which emphasize the need to conduct pharmacokinetic studies in specific species in their own environment rather than mere extrapolation.

The pharmacokinetic parameters of enrofloxacin are presented in Table 3 for single i.v. and oral bolus dose. The elimination rate constant was significantly higher ($p<0.01$) in i.v. when compared to oral bolus dose which was reflected by significantly lower ($p<0.01$) half life in i.v. (6.84±0.15h) than oral dose (10.57±0.35h). The half life in the present study is lower than reported values of 10.57±0.35h (i.v.) and 14.23±0.46h (oral) by (Anadon *et al.*, 1995). A comparable $t_{1/2\beta}$ of 5.56h (Knoll *et al.,* 1999) in chicken and 6.64h (Dimitrova *et al.,* 2007) in turkey after i.v. administration had been reported. (Silva *et al.*, 2006) recorded $t_{1/2\beta}$ of 14h after oral dose in chicken which was higher than the present study. In all the studies half life after oral dose was comparatively longer than i.v. route suggesting that the drug is eliminated faster after i.v. route. The half life in both the routes suggests that chicken eliminate enrofloxacin slowly and hence dosing interval may be prolonged.

Table 3: *Comparison of Mean Pharmacokinetic Parameters after Single IV and Oral Bolus Dose of Enrofloxacin in Broiler Chicken*

Parameters	Units	Mean±SE	
		Group I	Group II
β	h^{-1}	0.101**±0.002	0.065±0.002
$AUC_{0-\infty}$	μg.h.mL^{-1}	32.72*±1.15	25.35±1.92
$AUMC_{0-\infty}$	μg.h^2.mL^{-1}	282.83±15.45	405.06*±43.29
MRT	h	8.86±0.23	15.81**±0.54
MAT	h	-	6.95±0.54
$V_{d\ area}$/F	L.kg^{-1}	-	6.17±0.38
$V_{d\ area}$	L.kg^{-1}	3.04±0.09	4.69**±0.16
Cl_B/F	L.h^{-1}.kg^{-1}		0.41±0.03
Cl_B	L.h^{-1}.kg^{-1}	0.31±0.01	-
$t_{1/2\beta}$	h	6.84±0.15	10.57**±0.35
C_{max}	μg.mL^{-1}	-	1.63±0.12
t_{max}	h	-	3.58±0.61
F	%	-	77.47±5.86

*Significant (P<0.05)
**Significant (P<0.01)

The C_{max} obtained in the study was 1.63±0.12μg.mL^{-1} at 3.58±0.61h (t_{max}) and was concurrent with the findings of (El-Aziz *et al.,* 1997 (1.69μg.mL^{-1} at 2.52h); Knoll *et al.,* 1999 (1.9μg.mL^{-1} at 1.5h) and Silva *et al.,* 2006 (1.5 μg.mL^{-1} at 9h)) in chicken.

The mean AUC value was significantly higher ($p<0.05$) after i.v. when compared to oral administration (32.72±1.15 *vs* 25.35±1.92μg.h.mL^{-1}). (Anadon *et al.,* 1995) reported almost similar AUC of 34.51±1.30 and 22.26±0.69μg.h.mL^{-1} for i.v and oral administration. (Jakubowski *et al.,* 2010) reported lower value of 25.09μg.h.mL^{-1} for i.v. and (Silva *et al.*, 2006) reported higher value of 35.00μg.h.mL^{-1} for oral route. The AUC observed in the study are quite high which could be attributed to longer stay of drug in the body in both the routes and longer absorption phase in case of oral route. The MRT value was significantly low ($p<0.01$) in i.v. (8.86±0.23h *vs* 15.81±0.54h) than oral route which indicates the absorption phase was longer as evidenced by MAT of 6.95±0.54h in oral route.

The MRT of (Anadon *et al.,* 1995) was 9.65h for i.v and 15.40h for oral route and (Silva *et al.*, 2006) reported 15.64h which is almost similar to the present study.

The $V_{d\ area}$ obtained in this study after i.v. and oral route were 3.04±0.09L.kg^{-1} and 4.69±0.16L.kg^{-1}, respectively which differed significantly ($p<0.01$). Comparatively higher $V_{d\ area}$ of 4.31L.kg^{-1} (i.v.) and 5.94L.kg^{-1} (oral) were reported by (Anadon *et al.,* 1995) and 5.0L.kg^{-1} (i.v.) by (Knoll *et al.,* 1999) in chicken. Drugs with apparent volume of distribution greater than 1L.kg^{-1} were considered to be widely distributed in the body tissue (Baggot, 1977) and in the present study also enrofloxacin was found to be widely distributed in the body fluids and tissues of chicken as reflected by higher apparent volume of distribution.

The mean Cl_B of enrofloxacin obtained in the present study after i.v. was 0.31±0.01 L.h^{-1}.kg^{-1} and was comparable to (Anadon *et al.,* 1995) (0.29±0.01L.h^{-1}.kg^{-1}) and (Jakubowski *et al.,* 2010) (0.4L.h^{-1}.kg^{-1}) whereas (Knoll *et al.*, 1999) reported higher clearance of 0.62L.h^{-1}.kg^{-1} for the same dose. After oral administration the Cl_B/F was 0.41±0.03L.h^{-1}.kg^{-1} which was higher than the clearance reported by (Anadon *et al.,* 1995) (0.288±0.001L.h^{-1}.kg^{-1}).

The absolute bioavailability was 77.47±5.86 per cent and was comparable to 80.1 per cent reported in chicken (Bugyei *et al.,* 1999) and 80.35 per cent in turkey (Tansakul *et al.,* 2005). This value was higher than the reported bioavailability of 64 per cent (Anadon *et al.,* 1995), 59.6 per cent (El- Aziz *et al.,* 1997) in chicken, and 69.2 per cent (Dimitrova *et al.,* 2007) in turkey. The results confirmed that enrofloxacin was well absorbed after oral administration in chicken. However, the bioavailability calculations are only estimates, since different group of birds were used rather than a crossover design.

3.1. PK/PD Integration

The ultimate aim of pharmacokinetics study is to suggest appropriate dosage regimen which can produce clinical cure. Integration of PK variables such as AUC and C_{max} obtained in the study with PD variables *viz.* MIC (hypothetical values of 0.05, 0.125, 0.25, 0.5μg.mL^{-1}) revealed that the AUC/MIC was 236.37±7.78 and 155.62±9.63 for i.v. and oral route, respectively and C_{max}/MIC was 13.00±0.94 for oral route for microorganisms with MIC of 0.125μg.mL^{-1} (Table 4).

Table 4: *PK/ PD Integration of Enrofloxacin Based on Hypothetical MIC Values*

Ratio	MIC (μg.ml^{-1})	IV	Oral
AUC_{0-24}/MIC	0.05	590.92± 19.43	389.05±24.08
	0.125	236.37± 7.78	155.62±9.63
	0.25	118.18±3.89	77.81±4.82
	0.5	59.09±1.94	38.91±2.41
C_{max}/MIC	0.05	-	32.50±2.34
	0.125	-	13.00±0.94
	0.25	-	6.50±0.47
	0.5	-	3.25±0.23

In order to maximize clinical efficacy and minimize the development of resistance AUC/MIC>100-125 and C_{max}/MIC>8-12 should be achieved (Andes and Craig, 2002). In this study i.v route obtained AUC/MIC of 118.18±3.89 for MIC of 0.25μg.mL^{-1} but after oral route it was low. Hence based on AUC/MIC and C_{max}/MIC, enrofloxacin @ 10mg.kg^{-1} is sufficient to treat only moderately sensitive

organisms with MIC of 0.125μg.mL^{-1} after oral bolus administration whereas i.v route can treat organisms with MIC ≤0.25μg.mL^{-1}. For less sensitive organisms the dosage of enrofloxacin need to be increased as per the clinical situation.

4. Conclusion

The present study concludes that the bioavailability of enrofloxacin after oral administration was 77.47±5.86 per cent and desirable pharmacokinetic parameters could be attained. However the dose 10mg.kg^{-1} through oral route is sufficient to treat only moderately sensitive organisms with MIC≤0.125μg.mL^{-1} and dosage need to be increased based on the sensitivity of the microorganism.

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