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Pharmacokinetics and Tissue Residue Study of Levofloxacin Following Multiple Dose Intramuscular Administration in Poultry

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A B S T R A C T

The present study was undertaken to investigate the pharmacokinetics and tissue residue study of levofloxacin @ 10.0 $mg.kg^{-1}$ in poultry (*n*=16) following multiple (5) dose intramuscular (i.m.) administration. The concentration of levofloxacin in plasma and tissue of these animals were analysed by HPLC. Following multiple dose i.m. administration volume of distribution, clearance, AUC and elimination half-life after first dose was 5925.34 $ml.kg^{-1}$, 1401.3 $ml.h^{-1}.kg^{-1}$, 7.32 $h.\mu g.ml^{-1}$ and 2.97h, respectively and 5193.88 $ml.kg^{-1}$, 1663.16 $ml.h^{-1}.kg^{-1}$, 6.42 $h.\mu g.ml^{-1}$ and 2.30h after the last dose. Residue of levofloxacin was present in kidney, liver and muscle upto 72h post administration of levofloxacin with highest concentration of 0.14$\mu g.g^{-1}$in kidney. The limit of detection in tissue was 0.02$\mu.g^{-1}$.

Introduction

Levofloxacin, a second-generation fluoroquinolone, possesses excellent activity against Gram-positive, Gram-negative and anaerobic bacteria (Davis and Bryson, 1994; North *et al.*, 1998). Compared to other fluoroquinolones, ofloxacin and ciprofloxacin, it also has more pronounced bactericidal activity against organisms such as *Pseudomonas*, *Enterobacteriaceae* and *Klebsiella* (Klesel *et al.*, 1995). Levofloxacin is 100% available after oral administration (Chien *et al.*, 1997). The drug distributes well to target body tissues and fluids in the respiratory tract, skin, urine and prostrate and its uptake by cells makes it suitable for use against intracellular pathogens (Langtry and Lamb, 1998). However, it penetrates poorly into the CNS. Levofloxacin is metabolized in the liver to demethyl-levofloxacin and levofloxacin *N*-oxide. About 80% of a dose is found in the urine as unchanged drug and ≤5% as inactive metabolites (Langtry and Lamb, 1998). The pharmacokinetics of levofloxacin has been investigated in humans (Verho *et al.*, 1996; Amsden *et al.*, 1999; Chulavatnatol *et al.*, 1999; Gascon *et al.*, 2000), rabbits (Mochizuki *et al.*, 1994; Destache *et al.*, 2001), rats (Ito *et al.*, 1999) and guinea pigs

(Edelstein *et al*., 1996). However, there is no information available on the multiple dose kinetics of levofloxacin in poultry. The ever increasing use of quinolones, fluoroquinolones in poultry industry has caused their residual deposition in the poultry products resulting in the drug resistant bacteria. It has become a matter of foremost importance to screen the poultry birds of these residual antibiotics, down to the safer MRL's (Maximum Residue Limits). The present study was undertaken to determine the pharmacokinetics and tissue residue study of levofloxacin in poultry after multiple dose kinetics.

Materials and Methods

The pharmacokinetic and tissue residue study of levofloxacin was conducted in 16 poultry birds with an average weight of 1.0± 0.5kg. Levomac® (Levofloxacin hemihydrate infusion 0.5%) obtained from Macleods Pharm. Ltd. Ahmadabad was injected as multiple (5) dose i.m. at a dose rate of $10mg.kg^{-1}$ for 5 days at 24h interval in poultry birds. On 2^{nd}, 3^{rd} and 4^{th} days of drug administration, blood was collected by left and right brachial veins or from jugular vein immediately prior to drug administration and at 15 and 30 min post administration. On 1^{st} and 5^{th} day of drug administration, blood samples were collected at 0, 5, 10, 15 and 30 min & 1, 2, 4, 8, 12 and 24h following levofloxacin administration. Plasma was separated and stored at -30 C and analyzed by high-performance liquid chromatography (HPLC). All samples were analyzed within one week. The plasma proteins were removed via methanol precipitation; 200 μl plasma were mixed with 400 μl methanol and vigorously shaken. The precipitated proteins were removed via centrifugation at 12000×g for 5min. Subsequently, 20 μl of the supernatant were injected into the column. For tissue residue study four birds each were sacrificed at 24, 48, 72 and 96h post treatment. Blood samples were collected immediately prior to sacrifice. A postmortem examination was performed and samples of liver, kidney and muscle were collected frozen and stored at -40°C until analyzed. Drug extraction of levofloxacin from tissues was carried out by the method as described by Garcia–Ovando *et al.*, (1997). 4gm of thawed tissue with double amount of acetonitrile homogenize, triturated and was subjected to sonication at 10 amplitude microns for 30secs, with a pause of 5 seconds (a total of 15 cycles) by using ultrasonic tissue disintegration and centrifuged at 12000 for 15min and supernatant dried at 60^0C.

Clean up process of levofloxacin was done by the technique described by Telling and Sissons (1977) with certain modifications carried out using solid- phase extraction C_{18} cartridges. The dried eluate was reconstituted in 2ml of acetonitrile and loaded onto the conditioned C_{18} cartridges and allowed to pass through vacuum (20mmHg). The cartridges were washed with 2ml of acetonitrile. The eluate which was obtained after loading of cartridges was filtered through 0.22 µm filter paper. 20 µl of the sample thus obtained was injected into HPLC system for analysis. The solvents used during the chromatographic analysis of the drug were HPLC grade. The mobile phase consisted of water (80%) and acetonitrile (20%) with 0.3% of triethylamine and pH adjusted to 3.3 with phosphoric acid, with a flow rate of 0.7 ml /min to be detected at UV wavelength of 295 nm. The calibration curves of plasma were prepared with different concentrations between 0.025 and 10 μg/ml using blank poultry plasma. Pharmacokinetic analysis of plasma levofloxacin concentration versus time data was conducted by using WinNonLin Professional version 5.3 software package (Pharsight Corporation, Mountain View, California). The plasma concentration-time relationship was best estimated as a one compartment model for i.m. $Cp^{(t)} = Be^{-t}$.

Results and Discussion

Clinical examination of all animals before and after each trial did not reveal any abnormalities. No adverse reactions were observed after the multiple-dose i.m. administration of levofloxacin in the animals studied. A one-compartment model best represented the plasma concentration versus time data after i.m. administration of levofloxacin in poultry. The mean plasma concentration-time profiles of levofloxacin following multiple i.m. administrations of 10mg/kg b.wt after first and last dose are presented graphically in Figure 1. After first and last dose mean± SD values of pharmacokinetic parameters estimated from the curve fitting are shown in Table 1. Tissue residue concentration of levofloxacin (µg.g^{-1}) with their means following multiple (5) dose (10mg.kg^{-1}) i.m. administration is depicted in Table 2. The peak plasma concentration of levofloxacin was observed to be 1.42 and 1.50 µg.ml^{-1} at 30min after the first and last dose respectively. Thereafter, the plasma drug concentration decreased slowly to a minimum of 0.07 and 0.06 µg.ml^{-1} of first and last dose respectively. The elimination half-life (K_{10}_HL) after first and last dose of multiple dose study were 2.97h and 2.30h respectively. The volume of distribution and clearance of levofloxacin were estimated to be 5925.34mL.kg^{-1} and 1401.31 ml.h^{-1}.kg^{-1} respectively after first dose and 5193.88 mL.kg^{-1} and 1663.16 ml.h^{-1}. kg^{-1} after last dose. The mean area under curve (AUC) after first and last dose were 7.23±0.19 h. g.mL$^{-1.}$ and 6.42±0.34 h. g.mL^{-1} respectively. In the present study, it was observed that following multiple (5) dose i.m. administration in poultry, the levofloxacin in various tissues was high. The maximum residual concentration of levofloxacin was observed in kidney (0.14µg.g^{-1}) following multiple (5) dose i.m. administration in poultry after 24hr of last dose, which is comparatively higher than that of sarafloxacin (0.08 µg.g^{-1}; WHO, 1999) and lower than 0.3 µg.g^{-1} of flumequine (WHO, 2006). In liver the residual concentration of levofloxacin in the preent study was found to be 0.05 µg.g^{-1} which is less than that reported for sarafloxacin (0.08 µg. g^{-1}; WHO, 1999), and in muscles high concentration of levofloxacin was found as compared to 0.01 µg.g^{-1} of sarafloxacin (WHO, 1999).

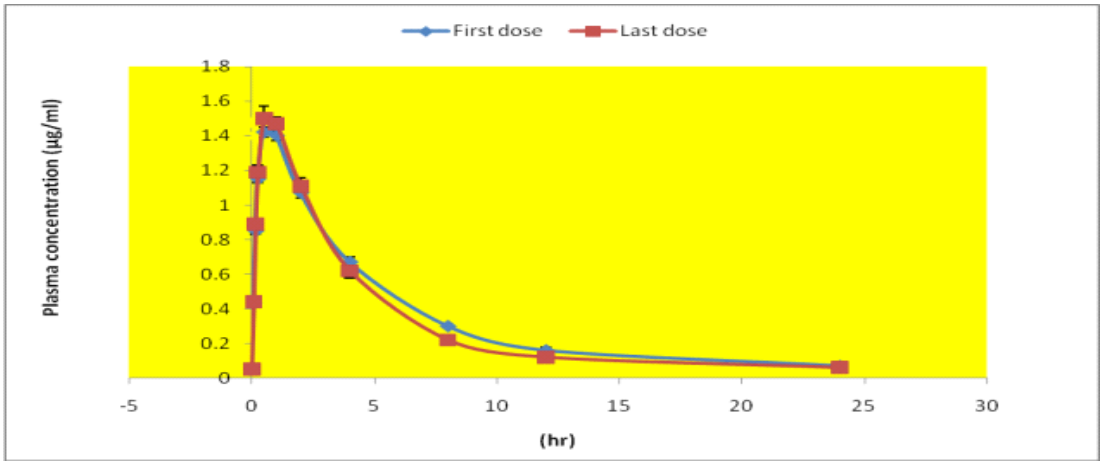

Fig.1 Plasma concentration (µg.ml^{-1}) of levofloxacin (Mean±S.E.) after first and last dose following multiple (5) dose (10 mg.kg^{-1}) intramuscular administration in poultry (n=16)

Table.1 Pharmacokinetic parameters of levofloxacin in plasma following multiple (5) dose (10mg.kg^{-1}) intramuscular administration in poultry (n=16)

Pharmacokinetic parameters	Units	Mean± S.E.	
		First dose	Last dose
V_F	ml.kg^{-1}	5925.34 ± 150.44	5193.88 ± 102.69
K_{01}	h^{-1}	4.63 ± 0.23	3.99 ± 0.22
K_{10}	h^{-1}	0.23 ± 0.01	0.31 ± 0.02
AUC	h.µg.mL^{-1}	7.23 ± 0.19	6.42 ± 0.34
K_{01}_ HL	h	0.15 ± 0.01	0.18 ± 0.01
K_{10}_ HL	h	2.97 ± 0.11	2.30 ± 0.12
CL_F	ml.h^{-1}.kg^{-1}	1401.31 ± 40.07	1663.16 ± 112.71
T_{max}	h	0.69 ± 0.02	0.71 ± 0.03
C_{max}	µg.ml^{-1}	1.44 ± 0.02	1.55 ± 0.03

Residual concentration (µg.g^{-1}) of levofloxacin in various tissues after 24h following multiple (5) dose (10 mg.kg^{-1}) intramuscular administration in poultry (n=4)

Tissue	Mean±S.E		
	24h	48h	72h
Liver	0.05	0.04	0.02
Kidney	0.14±0.03	0.09±0.02	0.04
Muscles	0.08±0.01	0.04	0.02

The residual concentration of levofloxacin after 48h post administration was found to be 0.09, 0.04 and 0.04 µg.g^{-1} in kidney, muscles and liver respectively. In kidney and muscle high concentration of levofloxacin was obtained than MRL of sarafloxacin in respective tissues i.e. 0.08 and 0.01µg.g^{-1} in kidney and muscles, respectively. After 72h of drug withdrawal, the residual concentration of levofloxacin in kidney declined to 0.04 µg. g^{-1}, whereas in liver and muscles equal tissue concentration of 0.02 µg. g^{-1} was observed. In liver (0.02±0.00 µg.g^{-1}), the concentration of levofloxacin was below maximum residual limit of sarafloxacin (0.08 µg.g-1; WHO, 1999). Levofloxacin could not be detected in liver, muscles and kidney after 96h post administration.

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