Safety level of levofloxacin following repeated oral administration in White Leg Horn layer birds

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Abstract

Levofloxacin is a fluorinated quinolone which has broad-spectrum antibacterial activity at low plasma/tissue concentration. The present study was designed to investigate safety of levofloxacin (10 mg/kg) after repeated oral administration at 12 hours interval for 14 days in layer birds (30-35 weeks old and weighing between 1.5-2.0 kg) and to determine tissue concentration of the drug following oral administration (10 mg/kg) for 5 days. Drug concentration in tissue was determined using High Performance Liquid Chromatography (HPLC). Repeated oral administration of levofloxacin in layer birds was found safe based on evaluation of haematological (Hb, PCV, TLC and DLC), blood biochemical (AST, ALT, AKP, ACP, LDH, BUN, Serum total protein, Serum albumin, Serum Creatinine, Blood glucose and Total bilirubin) and histopathology of liver, kidney and joint cartilage. Levofloxacin could not be detected in body tissues (liver and skeletal muscle) at 12 hours after the last administration.

Key words: Levofloxacin, Safety, Repeated oral administration, Layer birds

Introduction

Fluoroquinolones are gaining widespread acceptance in veterinary medicine as they have broad spectrum activity against Gram-negative and Gram-positive bacteria, mycopalasma, ricketsia as well as against bacteria resistant to other drugs (Brown, 1996). At present only few fluoroquinolones are used in veterinary medicine. Resistance of bacteria against fluoroquinolone is great threat for future survival of the fluoroquinolone drugs as an antibiotic class in veterinary medicine (Bakken, 2004). Levofloxacin is a newer molecule of third generation fluoroquinolones. It is active L - isomer of the racemate ofloxacin having twice antimicrobial activity than parent compound. Currently, it is extensively used in human medicine. However numbers of pharmacokinetic studies are being undertaken in domestic animals and poultry with a view to adopt this drug in veterinary medicine as well. It's spectrum of activity and pharmacokinetic properties favour its use in veterinary practice. Pharmacokinetics of levofloxacin have been studied in cow calves and poultry (Dumka, and Srivastava, 2006; 2007; Dumka, 2007, Ram et al., 2008; Patel, 2008). However, the data on safety of repeated oral administration of levofloxacin in poultry are lacking. Therefore, the present study was planned to evaluate safety of levofloxacin following multiple oral dose administration in White Leg Horn layer birds.

Materials and Methods

The present study was conducted on 14 white leg horn layer birds reared at Central Poultry Research Station, Anand Agricultural University, Anand, Gujarat, India. The birds were weighing between 1.50-2.00 kg. at 30-35 weeks of age. The birds were examined clinically to evaluate health status and to rule out the possibility of any diseases. They were kept in the individual layer cage and were maintained on standard antibiotics free layer ration. Water was provided ad libitum.

Eight layer birds were employed to assess safety of the drug. Birds were administered 10 mg/kg of levofloxacin at 12 hours interval for 14 days. Blood samples were withdrawn from wing vein into sterile heparinized (2ml) and non-heparinized (3ml) test tubes at 0 day (before drug administration) and on 3rd, 5th, 7th, 9th, 11th, 13th and 15th day for haematological [Hemoglobin (Hb), Packed cell volume (PCV), Total leukocytes count (TLC) and Diffrential leukocytes count (DLC)] and serum biochemical analysis (Aspartate aminotransferase (AST), Alanine transaminase (ALT), Alkaline phosphatase (AKP), Acid phosphatase (ACP), Lactate dehydrogenase (LDH), Blood urea nitrogen (BUN), Serum total protein, Serum albumin, Serum creatinine, Blood glucose and Total bilirubin), respectively. Haemoglobin was determined by Sahli's acid hematin method, PCV, TLC and DLC

as described by Schalm, (1967). Biochemical parameters were estimated using standard assay kits (Anamol Laboratories Pvt. Ltd., Palghar, India) with the help of automatic clinical serum biochemistry analyzer (Junior Selectra, Vital Scientific NV). Birds were observed for change in behaviour and palpated at joints to assess pain. Birds were scarified to collect tissue samples (liver, kidney and femoro-tibial and tibio-tarsal joint cartilages) for histopathological examination at 15th day. Tissues were processed by standard procedure and were stained with hematoxylin and eosin (H & E) for histopathological examination. Analysis of variance was used to detect differences between means for hematological and biochemical parameters. Statistical analysis of data was done by software SPSS (Version 12.0.1).

Six layer birds were employed to determine the accumulation of the drug in tissues following multiple oral administrations at dose rate of 10 mg/kg at 12 hours interval for 5 days. Birds were sacrificed at 12 hours after the last dose of the drug to collect the samples of liver and skeletal muscle. The drug concentration in tissue samples was determined by HPLC assay (Ishiwata et al., 2007).

Results and Discussion

After oral administration of the drug at the dose rate of 10 mg/kg body weight repeated at twelve hours interval, the values of hematological and serum biochemical parameters are presented in Table 1 and 2, respectively. No alterations in hematological and serum biochemical parameters have been found in the present study. It is in agreement with the non significant alterations in blood biochemical parameters reported in sixteen human volunteers following repeated oral administration of levofloxacin (Chien et al., 1997; Chien et al., 1998 and Chow et al., 2001). However increase in total bilirubin, AST, WBC and lymphocytes were observed in rats administered fandofloxacin (DW-116) at higher oral dose of 125 mg/kg (Kim et al. 2003). Residues of the drug were not detected in liver and kidney of birds after 12 h of administration of the drug.

Birds did not show change in behaviour and pain on palpation of joints during study period. All organs including liver and kidney were found to have normal colour, texture and consistency on gross necropsy examination of birds. On histopathological examination, no alterations at cellular level have been found in liver, kidney and joint cartilage. Gross and microscopic observations of the present study are in agreement with the result reported in rats treated with repeated oral dose of fandofloxacin (Kim et al. 2003). Similarly ciprofloxacin was also found safe in cow calves following repeated administration at dose rate 5 mg/kg body weight as no alterations were found in joint cartilage (Bhavsar et al., 2004). Transient neurological signs and lameness in horses were observed when administered with levofloxacin orally at the dose rate of 15 and 25 mg/kg for 21 days, but no alterations have been found after 7 days treatment (Alicia et al. 2000). Similarly, oral administration of newer fluoroquinolone (DW-2249) at dose rate of 30 mg/kg produced vomition, salivation, increased in serum cholesterol level, atrophy of thymus and testes in dogs but at low dose (10 mg/kg), no alterations were observed (Han et al., 2003).

In conclusion, following multiple oral administration, levofloxacin residue were not detected in the tissues. Results obtained in the present study indicate that levofloxacin is well tolerated following multiple oral administrations at 10 mg/kg body weight in layer birds.

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Table-1. Hematological parameters (Mean ± S.E.) after oral administration of levofloxacin (10mg/kg) in WLH layer birds

Hematological parameters	Days							
	0	3	5	7	9	11	13	15
Haemoglobin (g/dl)	9.93 ±0.34[a]	10.22 ± 0.10[a]	10.57 ± 0.22[a]	9.82 ± 0.39[a]	9.40 ± 0.39[a]	10.20 ± 0.39[a]	9.83 ± 0.33[a]	9.85 ± 0.36[a]
PCV (%)	29.83 ± 0.70[a]	29.17 ± 1.14[a]	29.33 ± 0.33[a]	29.33 ± 0.71[a]	29.34 ± 0.33[a]	29.17 ± 0.83[a]	29.67 ± 0.56[a]	28.83 ± 0.79[a]
TLC ($x10^3$ per cmm)	31.02 ± 0.49[a]	31.47 ± 0.39[a]	30.78 ± 0.83[a]	30.77 ± 0.29[a]	31.62 ± 0.37[a]	31.73 ± 0.44[a]	32.13 ± 0.59[a]	31.52 ± 0.52[a]
Heterophil (%)	28.50 ± 1.23[a]	29.67 ± 1.50[a]	29.67 ± 1.20[a]	29.00 ± 1.46[a]	32.17 ± 1.51[a]	30.00 ± 2.54[a]	28.17 ± 0.87[a]	30.67 ± 1.28[a]
Eosinophil (%)	3.17 ± 0.40[a]	2.67 ± 0.33[a]	2.83 ± 0.60[a]	2.83 ± 0.31[a]	2.67 ± 0.33[a]	2.17 ± 0.40[a]	3.00 ± 0.37[a]	3.00 ± 0.52[a]
Lymphocyte (%)	60.0 ± 1.56[a]	59.63 ± 1.51[a]	61.00 ± 1.57[a]	60.83 ± 2.02[a]	57.17 ± 2.12[a]	60.00 ± 2.31[a]	61.67 ± 0.56[a]	58.83 ± 1.40[a]
Monocyte (%)	8.17 ± 0.87[a]	7.83 ± 0.60[a]	6.83 ± 0.40[a]	7.33 ± 0.67[a]	8.00 ± 0.58[a]	7.83 ± 0.54[a]	7.17 ± 0.60[a]	7.50 ± 067[a]

Values having different superscript in the same row show significant difference (P< 0.05)

Table 2. Serum Biochemical parameters (Mean ± S.E.) after oral administration of levofloxacin (10mg/kg) in WLH layer birds

Parameters	Days							
	0	3	5	7	9	11	13	15
AST (IU)	196.83 ± 8.12[a]	211.83 ± 7.10[a]	216.00 ± 8.16[a]	205.83 ± 7.35[a]	203.00 ± 8.54[a]	208.83 ± 6.38[a]	199.00 ± 6.64[a]	196.17 ± 7.81[a]
ALT (IU)	24.33 ± 1.41[a]	25.67 ± 2.67[a]	26.17 ± 2.12[a]	25.00 ± 1.98[a]	25.67 ± 1.38[a]	27.83 ± 1.58[a]	25.50 ± 1.45[a]	24.50 ± 0.85[a]
AKP (IU)	63.17 ± 7.80[a]	69.40 ± 9.10[a]	64.84 ± 6.09[a]	64.00 ± 3.41[a]	59.50 ± 3.55[a]	61.17 ± 6.18[a]	64.17 ± 7.81[a]	64.84 ± 7.72[a]
ACP (IU)	5.88 ± 0.41[a]	6.07 ± 0.37[a]	5.81 ± 0.39[a]	5.69 ± 0.43[a]	5.74 ± 0.42[a]	5.84 ± 0.38[a]	5.89 ± 0.36[a]	5.87 ± 0.42[a]
LDH (IU)	349.32 ±6.39[a]	353.7 ±5.45[a]	353.1 ±4.75[a]	349.6 ±4.36[a]	350.4 ±5.84[a]	344.6 ±5.53[a]	347.2 ±5.61[a]	348.3 ±6.15[a]
Creatinine (mg/dl)	0.29 ±0.01[a]	0.30 ±0.02[a]	0.30 ± 0.01[a]	0.30 ±0.01[a]	0.29 ±0.01[a]	0.29 ±0.01[a]	0.29 ±0.01[a]	0.30 ±0.01[a]
Total Protein (gm/dl)	4.24 ±0.12[a]	4.20 ±0.16[a]	4.13 ±0.13[a]	4.09 ±0.13[a]	4.06 ±0.14[a]	4.27 ±0.15[a]	4.23 ±0.14[a]	4.23 ±0.11[a]
Albumin (gm/dl)	13.07 ±0.29[a]	13.04 ±0.18[a]	13.11 ±0.21[a]	13.07 ±0.24[a]	13.01 ±0.24[a]	12.96 ±0.29[a]	13.08 ±0.20[a]	13.12 ±0.28[a]
Glucose (mg/dl)	224.67 ±9.78[a]	228.5±8.90[a]	235.5 ±9.52[a]	230.0±6.57[a]	220.5±5.88[a]	222.6±9.04[a]	228.3 ±9.05[a]	224.0±7.79[a]
Total Bilirubin (mg/dl)	0.15 ±0.01[a]	0.16 ±0.02[a]	0.14 ±0.01[a]	0.14 ±0.01[a]	0.14 ±0.01[a]	0.17 ±0.01[a]	0.16 ±0.01[a]	0.15 ±0.01[a]
BUN (mg/dl)	4.38 ±0.20[a]	4.29 ±0.30[a]	4.36 ±0.21[a]	4.31 ±0.26[a]	4.44 ±0.24[a]	4.33 ±0.23[a]	4.32 ±0.19[a]	4.39 ±0.21[a]

Values having different superscript in the same row show significant difference (P< 0.05)

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