Effects of Concurrent Administration of Meloxicam on Pharmacokinetic Parameters of Enrofloxacin in Turkeys

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Abstract

The pharmacokinetic studies of enrofloxacin was conducted in eighteen turkeys (1 to 1.5 years age) weighing between 3 to 4 kg following single i.v. dose (10 mg/kg b.w.) alone and with meloxicam (1 mg/kg b.w.). Quantitative estimation of enrofloxacin and meloxicam was done by high performance liquid chromatography. The maximum concentration of drug in plasma (Cpmax) of enrofloxacin with meloxicam (8.42 ± 0.40 µg/ml) was not significantly different from that of enrofloxacin alone (9.68 ± 0.44 µg/ml). Pharmacokinetic parameters of enrofloxacin alone (C°p=10.38±0.43 µg/ml, t½β =2.73±0.12 h, MRT = 3.65±0.21 h, Clv 8.41±0.66 ml/kg/min, Vd = 1.95±0.08 L/kg) as compared to when it was administered with meloxicam (C°p=9.35±0.62 µg/ml, t½β =2.70±0.13 h, MRT =3.73±0.18 h, Clv 9.79±0.84 ml/kg/min, Vd =2.26±0.15 L/kg) in turkeys did not differ significantly.

The t½β of meloxicam with enrofloxacin (2.50±0.08 h) was significantly shorter as compared to meloxicam alone (3.03±0.18 h). Based on pharmacokinetic studies ENR may be injected at dose rate of 4.5 mg/kg i.v. at an interval of 20.38 h in turkeys.

Keywords: Pharmacokinetics; Enrofloxacin; Meloxicam; Interaction; Intravenous; Turkeys

Introduction

Enrofloxacin is 2nd generation fluoroquinolone developed exclusively for veterinary use [1,2]. It possess broad spectrum activity and is effective against many gram-negative organisms such as Escherichia coli, Salmonella, Klebsiella, Pasteurella, Proteus, Haemophilus, Complusbacctor and Pseudomonas and gram-positive bacteria like Streptococcus, Staphylococcus, Clostridium, Erysipelothrix and Mycoplasma. Enrofloxacin penetrates well into different tissues and has relatively slower elimination. Microbial resistance to their action does not develop rapidly [3].

Meloxicam is a safer non steroidal anti-inflammatory drug (NSAIDs) of oxicam class because it specifically inhibits cyclooxygenase -2 and has a superior gastrointestinal tolerability [4]. Its therapeutic index is six to twenty times more than other NSAIDs [5]. The meloxicam cause analgesia by suppressing generation of prostaglandin via inhibition of cyclooxygenase I and II enzyme [6]. It has no effect on platelet aggregation or renal prostaglandin synthesis and show sparing action on cyclooxygenase -1 [7,8]. Meloxicam is metabolized to four biologically inactive metabolites [9] and are equally excreted in urine and faeces. Meloxicam is used as anti-inflammatory, analgesic, antipyretic and prescribed with antibacterial agents. There are evidences that administration of two drugs together interact and can affect the pharmacokinetic parameters of each other and may affect the cure of diseases. It is possible that meloxicam may have some effect on the kinetic profile of enrofloxacin due to interaction which can increase or decrease the dose of each other. The pharmacokinetic parameters of ENR are available in other species of animals but there is paucity of such studies in turkeys. Therefore, the present work was designed to study the pharmacokinetic studies of enrofloxacin and its interaction with meloxicam in turkeys.

Materials and Methods

Experimental birds

Clinically 18 turkeys (either sex) of Instructional Varietal Bird farm, R.V.C. weighing between 3-4 kg (1 to 1.5 years) were used in this experiment. The birds were divided in to 3 groups (Group I- ENR alone, Group II – ENR + Meloxicam and Group III – Meloxicam alone) consisting of 6 birds in each group. The birds were kept in the experimental laboratory of Department of pharmacology & Toxicology and were provided standard ration and water ad libitum. The room temperature was maintained at 25ºC (±5ºC). The birds were dewormed with single oral dose of fenbendazole suspension @ 10 mg/kg b.w. 30 days prior to study. The protocol of the experiment was approved by Institutional Animal Ethics Committee of Ranchi Veterinary College, Ranchi.

Drugs used

(i) Enrofloxacin: Meriquin® 10% an injectable commercial preparation, containing 100 mg/ml enrofloxacin (w/v), marketed by Merind Pharmaceutical India was used. Enrofloxacin was injected at the dose rate of 10 mg/kg b.w. to each of the six turkeys by i.v. route.

(ii) Meloxicam: Melonex® an injectable commercial preparation, containing 5 mg/ml meloxicam, marketed by Intas Pharmaceutical Limited, Ahmedabad, Gujarat, India was used. Meloxicam was injected at the dose rate of 1 mg/kg b.w. to each of the six turkeys by i.v. route.

Collection of blood samples

Blood samples were collected from turkeys after i.v. administration.
of enrofloxacin alone and with meloxicam at predetermined time intervals at 0, 0.04, 0.08, 0.16, 0.25, 0.50, 0.75, 1, 2, 3, 4, 6, 8, 12, 24 and 48 h by wing venipuncture in heparinized test tube. Heparin was used at the dose rate of 20 µl (1% w/v solution) for 1 ml of blood. Plasma was separated by centrifugation at 3000 rpm for 20 minutes and was kept in refrigerator at 4ºC till analysis. The analysis was always done within 24 h of sample collection.

Estimation of enrofloxacin

Estimation of enrofloxacin was carried out by modified methods of Anadon et al. [16]. 0.5 ml of plasma was taken in a centrifuge tube and 1 ml of acetonitrile was added and mixed vigorously for 1 min by vortex mixer. The whole aliquot was centrifuged at 3000 rpm for 10 min and was filtered with Whatman no.1 filter paper (70 mm) and 20 µl of the filtrate was injected to the HPLC.

Estimation of meloxicam

Estimation of Meloxicam was carried out by modified method of Shukla et al. [11]. 0.5 ml of plasma was taken in a centrifuge tube and 0.5 ml of acetonitrile was added and mixed vigorously for 1 min by vortex mixture. The whole aliquot was centrifuged at 5000 rpm for 15 min, then 0.5 ml of supernatant was taken in centrifuge tube and 0.5 ml of HPLC grade water was added and mixed vigorously for 1 min by vortex mixture. The whole aliquot was centrifuged at 5000 rpm for 15 min, then it was filtered with Whatman no.1 filter paper (70 mm) and 20 µl of the filtrate was injected to the HPLC.

Preparation of mobile phase

(i) Enrofloxacin: 50 ml acetic acid, 100 ml acetonitrile, 50 ml methanol and 1 ml triethylamine (0.1%) were taken, then HPLC grade water was added to make 1000 ml and at last the pH of whole mixture was maintained at 3 by adding triethylamine.

(ii) Meloxicam: Water and acetic acid were taken in ratio of 99:1 v/v and from this 65% of mixture was taken and 35% of acetonitrile was added. At last HPLC grade water was added to make 1000 ml and at last the pH of whole mixture was maintained at 6 by adding triethylamine.

Experimental condition of apparatus

Cecil 4100 (Mtd. By Cecil instrumentation, Cambridge, England) liquid chromatograph coupled with variable wavelength UV/VIS detector attached with an integrator and LichroCART cartridge column was used. Injection of samples were done by 25 µl loop Hamilton syringe.

(i) Enrofloxacin: Mobile phase : As mentioned above.

A value : 280 nm
Flow rate : 1 ml/min
Temperature of oven : 40ºC

(ii) Meloxicam: Mobile phase : As mentioned above.

A value : 355 nm
Flow rate : 0.8 ml/min
Temperature of oven : 35ºC

Calculation of pharmacokinetic parameters

The pharmacokinetic parameters of enrofloxacin and meloxicam were calculated by computerized programme (Pharmkit) based on the formula [12-14].

Calculation of dosage regimen

The dose of enrofloxacin (mg/kg, b. w.) was calculated by standard method Shargel and Andrew [15].

\[ C_{P_{\text{max}}} = \frac{(\text{Dose}/Vd)}{1-e^{-k_r}} \]

Dosage interval of enrofloxacin was also calculated based on the method described by Shargel and Andrew [15].

\[ C_{P_{\text{max}}}/C_{P_{\text{min}}} = 1/e^{-k_r} \]

Statistical analysis

The statistical comparison of important pharmacokinetic parameters were done as per statistical method of Snedecor and Cochran [16]. Quantitative data were analysed, using the independent t-test.

Result

The comparative mean pharmacokinetic parameters of enrofloxacin with and without meloxicam are presented in Table 1. The mean value of \( C_{P0} \) of ENR alone and with meloxicam were 10.38±0.43 µg/ml and
Kinetic Parameters Without meloxicam With meloxicam t-value
A(µg/ml) 5.59±0.26 4.97±0.29 1.48 NS
B(µg/ml) 4.79±0.25 4.38±0.35 0.88 NS
C’p(µg/ml) 10.38±0.43 9.35±0.62 1.26 NS
α(h⁻¹) 2.22±0.21 2.07±0.51 2.14 NS
β(h⁻¹) 0.26±0.01 0.26±0.01 0.28 NS
t_α(h) 0.27±0.05 0.23±0.05 0.51 NS
t_β(h) 2.73±0.12 2.70±0.13 0.15 NS
AUC(mg/L.h) 20.51±1.47 17.72±1.40 1.26 NS
AUMC(mg/L.h) 76.56±8.99 66.94±7.16 0.76 NS
MRT(h) 3.65±0.21 3.73±0.18 0.27 NS
ClB(ml/kg/min) 0.81±0.04 0.87±0.08 0.57 NS
Vdarea(L/kg) 0.21±0.01 0.19±0.02 0.87 NS
K12(h⁻¹) 2.50±0.72 1.98±0.38 0.58 NS
K21(h⁻¹) 2.28±0.64 2.11±0.32 0.21 NS
K2(h⁻¹) 0.47±0.03 0.56±0.02 2.14 NS
T/P 0.91±0.06 0.94±0.06 0.28 NS

NS=Non Significant

Table 1: Comparative mean pharmacokinetic profile of ENR after single dose (10mg/kg) i.v. administration with and without MLX (1mg/kg, i.v.) in turkeys.

Kinetic Parameters Without ENR With ENR t-value
A(µg/ml) 5.61±0.42 6.43±0.55 1.07 NS
B(µg/ml) 4.38±0.43 5.39±0.56 1.32 NS
C’p(µg/ml) 9.99±0.74 11.81±1.08 1.27 NS
α(h⁻¹) 4.80±1.45 4.38±0.69 0.24 NS
β(h⁻¹) 0.23±0.01 0.28±0.01 2.57* t½α(h) 0.36±0.17 0.11±0.03 1.30 NS
Kα(h⁻¹) 0.23±0.01 0.28±0.01 2.57* t½β(h) 3.03±0.18 2.50±0.08 2.39* AUC(mg/L.h) 20.74±0.92 20.21±1.96 0.22 NS
AUMC(mg/L.h) 81.87±4.06 69.67±7.31 1.33 NS
MRT(h) 3.95±0.10 3.44±0.11 3.19** Clα(mg/kg/min) 0.81±0.04 0.87±0.08 0.57 NS
Clβ(mg/kg/min) 0.21±0.01 0.19±0.02 0.87 NS
Vdαβ(L/kg) 2.50±0.72 1.98±0.38 0.58 NS
Vdαβ(L/kg) 2.28±0.64 2.11±0.32 0.21 NS
Vdαβ(L/kg) 0.47±0.03 0.56±0.02 2.14 NS
T/P 0.91±0.06 0.94±0.06 0.28 NS

NS=Non Significant, *P<0.05,**P<0.01

Table 2: Comparative mean pharmacokinetic profile of MLX after single dose (1 mg/kg) i.v. administration with and without ENR (10 mg/kg, i.v.) in turkeys.

The mean comparative pharmacokinetic parameters of meloxicam in plasma of turkeys with and without enrofloxacin are presented in Table 2. The mean value of C’p in turkeys without enrofloxacin was 9.99±0.74 µg/ml and with enrofloxacin was 11.81±1.08 µg/ml in turkeys with enrofloxacin. The mean value of β of meloxicam without enrofloxacin was 0.23±0.01 h⁻¹ and with enrofloxacin was 0.28±0.01 h⁻¹ in turkeys. The mean value of t½β of meloxicam in turkeys without enrofloxacin was 3.03±0.18 h and this value was 2.50±0.08 h in turkeys with enrofloxacin (see supplementary data).

The mean values of kinetic parameters of meloxicam i.e. AUC, MRT, Clα and Vdαβ were 20.74±0.92 mg/L.h, 3.95±0.10 h, 3.44±0.11 h and 3.19±0.11 h respectively in turkeys with meloxicam. The values of above parameters were 20.21±1.96 mg/L.h, 3.44±0.11 h, 0.87±0.08 mg/L/h and 0.21±0.02 mg/L/h respectively in turkeys with meloxicam.

The maximum plasma concentration (Cp max) of enrofloxacin with meloxicam (8.42±0.40 µg/ml) was not significantly different from that of enrofloxacin alone (9.68±0.44 µg/ml). However, the effect of concurrent administration of enrofloxacin with meloxicam in febrile turkey could not be studied. Therefore, the further study on enrofloxacin in febrile turkey especially with meloxicam is required to pin-point its effect on the plasma levels. Tansakul et al. [17], similar to the finding of this study reported Cp max of enrofloxacin (11.49±1.17 µg/ml) after single dose (10 mg/kg, b.w.) after i.v. administration in healthy duck. It was observed that the plasma concentrations obtained after i.v. administration in turkeys with meloxicam (0.30±0.04 µg/ml) and without meloxicam (0.30±0.03 µg/ml) were much higher than the reported MIC values (0.01 to 2.0 µg/ml) [18,19]. Therefore, it is obvious...
that enrofloxacin may combat infections caused by various susceptible pathogens in turkey if given in emergent conditions by i.v. route.

The mean Cp of meloxicam with enrofloxacin (9.36±0.44 µg/ml) in turkeys was not significantly different from that observed in meloxicam alone (9.30±0.43 µg/ml). Result obtained indicate that enrofloxacin does not change the pharmacokinetic profile of meloxicam when both are given together.

The mean Cp of enrofloxacin with meloxicam (9.35±0.62 µg/ml) in turkeys was not significantly different from that observed after enrofloxacin alone (10.38±0.43 µg/ml). Ranjan [20] also reported that meloxicam at dose rate of 0.5 mg/kg b.w. i.v. along with ceftizoxime (25 mg/kg, b.w.) in healthy sheep did not show any significant change in the Cp of ceftizoxime. The results of Cp evidenced that enrofloxacin may be used alone or with meloxicam in emergent diseases of turkey.

The t½β of enrofloxacin with meloxicam (2.70±0.13 h) was almost similar to that without meloxicam (2.73±0.12 h) in turkeys. The results showed that t½β of enrofloxacin with and without meloxicam in healthy turkeys did not differ significantly. Kanemaki et al. [21] reported almost similar t½β (3 h) in dog.

The mean MRT value of enrofloxacin with meloxicam (3.73±0.18 h) did not differ significantly when enrofloxacin was administered alone (3.65±0.21h). However, Ahmed et al. [22] reported a higher MRT (8.82±0.21h) of enrofloxacin after single dose (7.5 mg/kg, b.w.) i.v. administration in yak.

The mean Cl value of enrofloxacin with meloxicam (9.79±0.84 ml/kg/min) did not differ significantly as compared with enrofloxacin alone (8.41±0.66 ml/kg/min). It is obvious from the results obtained in this experiment that similar Cl values of enrofloxacin with and without meloxicam in turkeys could produce similar initial plasma levels after i.v. administration in turkeys. Single dose kinetics of rifampicin, isoniazid as well as their combination dosage forms as tablet and capsule has been carried out in humans. Significantly greater rate and extent of absorption was observed from rifampicin capsule alone as compared to the rifampicin levels from combination dosage forms [23].

The mean Vd of enrofloxacin with meloxicam (2.26±0.15 L/kg) did not differ significantly as compared with enrofloxacin alone (1.95±0.08 L/kg). Result indicated that meloxicam did not hamper the pharmacokinetic profile of enrofloxacin when both were given together. Ahmed et al. [22] reported Vd of (5.78±0.84 L/Kg) of ENR after single dose (7.5 mg/kg, b.w.) i.v. administration in yak. Similar Vd value (2.5±0.20 L/kg) has also been reported for ENR after i.v. administration (5 mg/kg) in ostrich [24]. The high Vd value of ENR with meloxicam obtained after i.v. administration (2.26±0.15 L/kg) indicated its good penetration into wide range of tissue in turkeys. The apparent value of volume of distribution of ENR in birds is reported to be variable, ranging between 1.49 to 3.9 L/kg.

The t½β of meloxicam with ENR (2.50±0.08 h) was significantly (p<0.05) shorter in healthy turkeys as compared to meloxicam alone (3.03±0.18). Baert and Backer [25] reported t½β of 3.21h after i.v. administration (0.5 mg/kg, b.w.) in chicken.

The mean MRT value of meloxicam with enrofloxacin (3.44±0.11 h) was significantly (p<0.01) lower in healthy turkeys as compared to meloxicam alone (3.95±0.10 h). Baert and Backer [25] also reported MRT value (4.41 h) of meloxicam similar to this study in chicken after i.v. administration (0.5 mg/kg, b.w.).
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