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Pharmacokinetics and Withdrawal Times of Enrofloxacin in Ducks

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ABSTRACT

The pharmacokinetic properties of enrofloxacin (EFX) were investigated in healthy ducks following a single administration of EFX with a dose of 10 mg/kg of body weight by intravenous (i.v.), intramuscular (i.m.), subcutaneous (s.c.) or oral (p.o.) route. The plasma concentration-time curve was analyzed using a two compartment model. Mean peak plasma concentration of EFX was 11.49 ± 1.17, 5.65 ± 0.36, 4.99 ± 0.87 and 4.87 ± 0.69 mg/ml after i.v., i.m., s.c. and p.o. administration, respectively. After a single i.v. administration, the pharmacokinetic parameters were found as follow; the elimination half-life ($t_{1/2}\beta$) = 6.47 ± 2.85 h, the elimination rate constant (K_{el}) = 0.70 ± 0.06 h^{-1},the apparent volume of distribution $V_{d(area)}$ = 1.30 ± 0.22 L/kg and the total body clearance (Cl_B) = 0.89 ± 0.07 L/kg/h. Difference enrofloxacin bioavailability following i.m., s.c. and p.o. administration were 98.77 ± 0.05 %, 85.11 ± 2.71 % and 80.35 ± 0.29%, respectively. The results of pharmacokinetic properties of EFX in ducks should be provided with the dosage regimen, preslaughter withdrawal times and maximum residue limits for ducks.

Key words: pharmacokinetic, withdrawal time, antibiotic, enrofloxacin, duck species

INTRODUCTION

Enrofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[4-ethyl-1-piperazinyl] -3-quinoline carboxylic acid) is an antimicrobial substance which belongs to the fluoroquinolones groups. This agent reportedly has excellent activities against a wide range of aerobic gram-negative bacteria. It is also active against gram-positive bacteria and *Mycoplasma spp*. Therefore, EFX is commercialized for animal use and potential therapeutic application for many types of infection (García-ovando *et al*.,1999). Similar to that of other quinolones, these compounds act on inhibition of DNA gyrase and exhibit a bactericidal and mycoplasmacidal activity at low concentrations. The efficacy of EFX reportedly inhibits *in vivo* replication of certain organisms that are resistant to antibacterial substances i.e., beta-lactam antibiotics, aminoglycosides, tetracyclines, folic acid antagonists and macrolides (Anadón *et al*., 1995). Limited information is available on disposition, metabolism and safety of EFX use in commercial ducks. The objective of the present study was to investigate the fundamental pharmacokinetic value of EFX on ducks following

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intravenous (i.v.), intramuscular (i.m.), subcutaneous (s.c.) and oral (p.o.) administration. Thereafter, the proper therapeutic regimen of EFX should be concerned for ducks.

MATERIALS AND METHODS

Animals

Healthy ducks of an average 1.09 ± 0.24 kg body weight, without previous treatment, were used in the study. Ducks were separated into four groups (30 ducks per group). The animals were fed with a commercial standard diet that was free from any chemotherapeutics three times per day. Water supply was provided *ad libitum*. Throughout the study they were housed in the animal cage at Division of Experimental Animal, Faculty of Veterinary Medicine, Kasetsart University.

Drug administration and sample collection

Commercial enrofloxacin containing 50 mg/ml (Baytril® 5% sterile solution, Bayer AG, Leverkusen,Germany) was prepared for i.v., i.m., s.c. and p.o. administrations at the same dose of 10 mg/kg body weight for each duck. Randomized 2.5 ml of heparinized blood were taken from the brachial vein in the following preset times:0.0, 0.15, 0.30, 1, 2, 3, 4, 5, 6, 8, 10, 12, 20, 24, 30, 48, 54, 72, 78 and 92 h. Blood samples were collected and centrifuged (3000 X g) for 15 min to collect the plasma (García-ovando *et al.*,1999), placed in a 1.5 ml Eppendorf vial (Laboratory Product, Inc., Rochester, NY.) and stored at -20°C until analysis.

Method of analysis

The concentration of EFX was analyzed using a microbiological diffusion method (Bennett *et al.*,1966; Anhalt,1985; Limpoka,1992). The method used *Escherichia coli* ATCC 25922 (Scientific and Technology Institute of Thailand) as test organisms. Standard dose-response curves were obtained using buffer EFX solution. The motten agars were prepared by inoculated with the organisms in broth. Then the medium was poured 32 ml into each 10 × 15 cm glass plate. After hardened, 10 mm diameter wells were punched 8 holes per plate. Then the plasma samples and standard control (2 holes) were examined. The samples were allowed to diffuse for 45 min at room temperature prior to incubation for 24 h at 37°C. Thereafter, the inhibition zone of the standard preparations and samples were measured using a caliper vernia. The concentrations were recorded from plots of log concentration plus zone diameter of plasma.

Calculation of pharmacokinetic parameters

The pharmacokinetic values of EFX on plasma concentrations after a single i.v. administration were evaluated by a semilogarithm modified standard technique. A bi-exponential equation was selected for all ducks having been given the drug by the i.v. route and consequently the data were described by a two-compartment open model based on the criteria of improvement in the sum square by plotting of residuals. The following pharmacokinetic parameters were obtained according to the conventional equations previously described by Baggot (1977), Limpoka (1992) and Craigmill *et al.* (1994).

The following equations were used to obtain these pharmacokinetic parameters for a two-compartment pharmacokinetic model.

$t_{1/2}\alpha = \ln 2/\alpha$

$t_{1/2}\beta = \ln 2/\beta$

$K_{21} = A(\beta) + B(\alpha)/ A+B$

$K_{el} = (\alpha)(\beta)/ K_{21}$

$K_{12} = \alpha+\beta- K_{21} - K_{el}$

$V_{d(area)} = \text{Dose}/C_p^o$

$\text{AUC} = (A/\alpha) + (B/\beta)$

$F = AUC_{other} / AUC_{i.v}$

$Cl_B = (K_{el})\ (V_c)$

The term of Cp^o is the extrapolated plasma concentration to determined the zero- time profile. B was calculated from the elimination phase (B-slope). A was calculated by the residual method

(O'Flaherty, 1981). The a and b are hybrid rate constants describing the initial and terminal decline in plasma concentration and are composed of the microrate constants (K_{12},K_{21}) of the model. The $t_{1/2}\alpha$ (distribution half-life), $t_{1/2}\beta$ (elimination half-life), AUC (area under the curve), $V_{d(area)}$ (apparent volume of distribution during the post-distribution phase), Bioavailability and Cl_B (total body clearance) were calculated.

Statistic analysis

The pharmacokinetic parameters were calculated by CA-Cricket Graph III, version 1.5J, Computer Associates Inc., NY., U.S.A. Statistical analysis of data was performed using Microsoft Excel, Window XP.

RESULTS

After a single i.v. administration of 10 mg/kg of body weight of EFX in ducks, the mean ± SD pharmacokinetic parameters were calculated and described by a two-compartment open model. Distribution half-life ($t_{1/2}a$) was 0.60 ± 0.02 h, whereas the elimination half-life($t_{1/2}\beta$) was 6.47 ± 2.85 h. Table 1. presents the pharmacokinetic parameters.

Comparison of the mean ± SD plasma concentration-time profile of EFX at various routes are shown in Table 2. and Figure 1. EFX was absorbed rapidly. Concentrations of EFX peaked within 30 min by i.m. administration while the peak levels of s.c and p.o. administration were found within 1 h. However, these levels were higher than the therapeutic level (Anonymous; 1987).

DISCUSSION

Pharmacokinetic variables of EFX after the i.v. administration were best described by a two-compartment open model, with a rapid distribution phase ($t_{1/2}\alpha$ = 0.6 h) and a moderately prolong elimination phase ($t_{1/2}\beta$ = 6.47 h).

Because of limited reports of fluoroquinolones in ducks, the time to maximum concentration (t_{max}) differed among enrofloxacin, ciprofloxacin and norfloxacin in chicken were applied as reference. The significant differences ($p<0.05$) were found that the t_{max} of ciprofloxacin (0.42 ± 0.08 h) (Atta and Sharif,1997) was reached more rapidly than that of enrofloxacin (1.64 ± 0.04 h) (Anadón *et al.*,1995) and norfloxacin (1.99 ± 0.17 h) (Laczay *et al.*,1998) after oral administration. In addition, the peak plasma concentration (C_{max}) of ciprofloxacin was the highest (4.67 ± 0.33 µg/ml),which was higher than that of the enrofloxacin (2.44 ± 0.64 µg/ml) and norfloxacin (1.46 ± 0.18 µg/ml).

Table 1 Pharmacokinetic data (mean ± SD) for enrofloxacin determined following intravenous administration at a single dose of 10 mg/kg of body weight in ducks.

Pharmacokinetic parameters (units)	Enrofloxacin
Cp^o (µg/ml)	15.73 ± 2.60
A (µg/ml)	14.67 ± 3.38
α (h^{-1})	1.16 ± 0.03
B (µg/ml)	1.35 ± 0.90
β (h^{-1})	0.13 ± 0.07
$t_{1/2}\alpha$ (h)	0.60 ± 0.02
$t_{1/2}\beta$ (h)	6.47 ± 2.85
K_{12} (h^{-1})	0.37 ± 0.003
K_{21} (h^{-1})	0.22 ± 0.13
K_{el} (h^{-1})	0.70 ± 0.06
$V_{d(area)}$ (L/kg)	1.30 ± 0.22
Cl_B (L/kg/h)	0.89 ± 0.07
$Bioavailability_{i.m.}$ (%)	98.77 ± 0.05
$Bioavailability_{s.c.}$ (%)	85.11 ± 2.71
$Bioavailability_{p.o.}$ (%)	80.35 ± 0.29

Note: Pharmacokinetic parameters of EFX were determined by a two-compartment pharmacokinetic model.

A similar kinetic profile was also observed in chickens after the i.v. administration, the biphasic nature of the plasma concentration-time curve has been reported for EFX (Anadón *et al*.,1995;García-ovando *et al*.,1999). In the present study, the elimination half-life (6.47 ± 2.85 h) was also higher than that recorded in healthy dogs (3.4 h), cattle (1.7h), sheep (3.7 h), horses (5.0 h) and pigs (5.5 h) (Baggot,2001). However, this parameter was lower than that previously reported in chickens (6.99 ± 0.48 h) (García-ovando *et al*.,1999).

Fluoroquinolones are lipid-soluble chemical agents, and their typical Vd values are 2-4 L/kg (Brown,1996). Nevertheless, lower Vd values i.e., 1.94 ± 0.14 L/kg have been reported for EFX in chickens (García-ovando *et al*.,1999)

Table 2 Mean ± SD plasma concentrations of enrofloxacin in ducks following i.v., i.m., s.c. or p.o. administration at a single dose of 10 mg/kg of body weight.

Hours after dosing	Plasma concentrations (mg/ml)			
	i.v.	i.m.	s.c.	p.o.
0.15	11.49 ± 1.17	5.65 ± 0.36	4.99 ± 0.87	4.87 ± 0.69
0.30	10.46 ± 1.85	8.97 ± 1.33	6.48 ± 1.07	5.55 ± 0.70
1.00	5.59 ± 0.28	7.99 ± 0.63	8.29 ± 0.62	7.61 ± 1.20
2.00	4.00 ± 0.25	7.60 ± 0.68	7.19 ± 1.12	5.99 ± 0.34
3.00	2.08 ± 0.36	6.76 ± 0.99	7.06 ± 0.58	5.74 ± 0.60
4.00	1.97 ± 0.15	5.72 ± 0.58	6.67 ± 0.73	4.62 ± 0.39
5.00	1.06 ± 0.16	3.90 ± 0.35	4.44 ± 0.68	4.43 ± 0.59
6.00	0.86 ± 0.19	3.57 ± 0.41	2.95 ± 1.13	3.99 ± 0.73
8.00	0.47 ± 0.07	1.51 ± 0.17	1.46 ± 0.10	2.28 ± 0.21
10.00	0.43 ± 0.04	1.43 ± 0.17	1.42 ± 0.25	2.06 ± 0.43

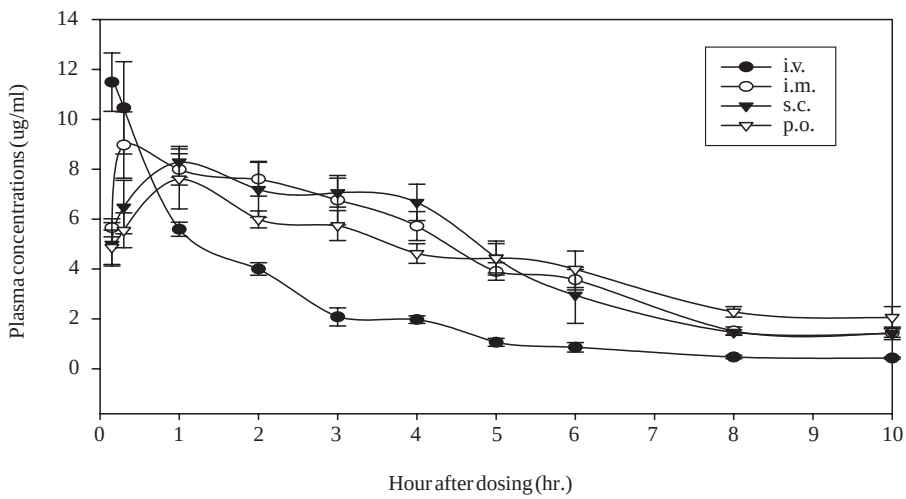

Figure 1 Comparative mean plasma concentration-time profile of enrofloxacin (EFX) following single i.v.,i.m.,s.c.and p.o. administrations of 10 mg/kg b.w. in ducks.

The mean ± SD bioavailability of EFX in ducks was 98.77 ± 0.05% after the i.m. administration, therefore it is likely that the dose of EFX was almost completely absorbed. The bioavailability value of the i.m. administration was also higher than those of the s.c. (85.11 ± 2.71%) and p.o. (80.35 ± 0.29%). Moreover, the drug was detected and remained in the plasma up to 20 h after the s.c. and i.m. administrations while it was up to 24h after the i.v. and p.o. administrations.

In conclusion, The biphasic nature of plasma concentration-time curve suggested that a two-compartment pharmacokinetic model would provide an accurate description of pharmacokinetic behaviors. The pattern of plasma concentration-time profiles between EFX and the other fluoroquinolones were identical following i.m., s.c. or p.o. administration . According to the results of this study a dose of 10 mg/kg body weight of enrofloxacin in ducks may be appropriate for the routes investigated. However, the tissue residues should be further determined by an HPLC assay to get insight into the tissue uptake and the proper withdrawal times of EFX in ducks.

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