International Journal of Poultry Science 9 (3): 273-277, 2010
ISSN 1682-8356

Comparative Pharmacokinetics/Pharmacodynamic Modeling on Three Brands of 10% Enrofloxacin Oral Formulations in Broiler Chickens

Fidelis Aondover Gberindyer[1], Noel Wannang[2] and Chinedu Adive Akwuobu[3]
[1]Department of Veterinary Physiology, Pharmacology and Biochemistry, University of Agriculture, Makurdi, Nigeria
[2]Department of Pharmacology, University of Jos, Jos, Nigeria
[3]Department of Veterinary Pathology and Microbiology, University of Agriculture, Makurdi, Nigeria

Abstract: A comparative plasma pharmacokinetics/Pharmacodynamic modeling of enrofloxacin following administration of three brands of 10% enrofloxacin was studied in healthy broiler chickens using a randomized and parallel design. Pre-treatment and post-treatment samples were obtained from brachial or right jugular veins after having administered 20 mg/kg b.w of enrofloxacin at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h. Plasma samples were analyzed for enrofloxacin concentration by a simple agar disk diffusion microbiological assay. Selected pharmacokinetics parameters were calculated using a non-compartmental model. There was a significant difference in the plasma concentration-time and pharmacokinetics profiles ($p<0.05$) of the three brands. But the plasma concentrations of enrofloxacin exceeded the MIC_{90} for most pathogenic bacterial organisms in poultry in all the groups. The PK/PD integration (C_{max}/MIC_{90}) values, 16.67, 15.17 and 10.5 h were obtained in animals administered conflox®-vet, kenflox® and pulmotryl® formulations respectively. This correlates with high efficacy and reduced chances for the development of resistant pathogenic bacterial organisms following oral administration of these brands of enrofloxacin oral formulations in broiler chickens.

Key words: Pharmacokinetic, pharmacodynamic, enrofloxacin, brands, resistance, chickens

INTRODUCTION

Enrofloxacin, a Fluoroquinolone, bactericidal and broad spectrum antibiotic is used exclusively in Veterinary medicine for the treatment of septicemia, respiratory tract, urinary tract, skin, soft tissues, bone and joint infections (Sanjib *et al*., 2005). In many countries enrofloxacin is being used as the routine choice to treat almost any bacterial disease in poultry (Sumano and Gutierrez, 2000; Sumano and Gutierrez, 2001). Since Fluoroquinolones generally exhibit concentration-dependent effect, its activity increases with increasing concentrations at its sites of action (Craig, 1993; Mouton and Tulkens, 2005). Knowledge of disposition kinetic of antibacterial agents alone is inadequate in predicting their therapeutic efficacies. Thus, a Pharmacokinetics/ Pharmacodynamics (PK/PD) integration is critical in relating the exposure (PK) and response (PD) to drug, which could be desirable or undesirable (Reiko *et al*., 2006). It also establishes a mathematical and theoretical link between PK and PD and helps better predict drug action (Lakshmi, 2006).

The pharmacokinetic parameters most frequently used for PK/PD modeling in concentration-dependent antimicrobials are those which reflect an increase in drug concentration and exposure, C_{max} and AUC (Baggot, 2001; Mouton and Tulkens, 2005). The biomarkers commonly linked to clinical outcome of antimicrobials are the ratio of peak plasma concentration of drug to minimum inhibition concentration, C_{max}/MIC; the ratio of 24-h area under the plasma concentration-time curve to minimum inhibition concentration, AUC_{0-24}/MIC and the duration of time that plasma levels exceed the minimum inhibition concentration, T >MIC (Baggot, 2001; Marie, 2007). Clinical response usually correlates with AUC_{0-24}/MIC and C_{max}/MIC for concentration-dependent antimicrobial agents, but the latter, C_{max}/MIC is found to be relatively more important for Fluoroquinolones where the ratio of about 5-10 has been associated with high efficacy and lower incidence of developing bacterial resistance (Baggot, 2001). Other modeling studies revealed that survival of the host and minimized risk of the emergence of resistant bacterial strains is linked to C_{max}/MIC when the ratio is equal or greater than 10 (Meinen *et al*., 1995; Dowling *et al*., 1995; Mouton and Tulkens, 2005).

Because of high prevalence of enrofloxacin sensitive bacterial infections in poultry, scarcity and high cost of the pioneer product (Baytril®), there has been a tremendous increase in the use of other brands of enrofloxacin. With increasing availability and use of generic enrofloxacin products from different pharmaceutical companies, practitioners are faced with the dilemma of therapeutic failures and side effects following the use of some of these arrays of

Corresponding Author: Chinedu Adive Akwuobu, Department of Veterinary Pathology and Microbiology, University of Agriculture, Makurdi, Nigeria

multisource products in the market. Since these clinical conditions results in great economic losses to farmers and the pioneer formulations and few brands have severally proven effective, there is a need to investigate the main surrogate efficacy marker, C_{max}/MIC using MIC_{90} against the most common pathogenic bacterial organisms in poultry (Sanjib *et al*., 2005).

MATERIALS AND METHODS

Study products: Conflox®-vet (10% enrofloxacin) from India (Batch No: 70002, Exp. 06-2012); kenflox® (10% enrofloxacin) from Holland (Batch No: 0811703, Exp. 03-2011) and pulmotryl® (10% enrofloxacin and 1% bromhexine hydrochloride) from Jordan (Batch No. 08-022, Exp. 06-2012). Pure enrofloxacin (≥98%) from Sigma- Aldriech, USA was used as a standard. Nutrient agar by Lab M, USA and *Escherichia coli*, NCTC10418 from Zaria were used as the media and test microorganism respectively.

Experimental subjects: Thirty six broiler chickens, 8 weeks old, weighing 2.5-3.0 kg body weight (b.w) were used. They were purchased as day old chicks from a hatchery in Ibadan, Nigeria and managed under deep litter system. They were vaccinated against most common infectious poultry diseases. The feed was formulated without inclusion of drugs. At 5 weeks old, the apparently healthy chickens were separated and allowed to acclimatize in the experimental environment for three weeks during which no drug, except multivitamins was administered to them.

Experimental design: A randomized, single oral dose, parallel method was adopted. The animals were assigned to three groups; A, B and C of 12 animals each. Feeds and water were withdrawn 8 and 2-h respectively before drug administration. This was to reduce absorption variability due to drug-feed interaction and over dilution of the drug respectively (Randandt *et al*., 1992). Animals in groups A, B and C were weighed individually and administered by gavage conflox®-vet, kenflox® and pulmotryl® brands of 10% enrofloxacin oral formulations respectively at a dose level of 20 mg/kg b.w. The animals were monitored and those that regurgitated were excluded from the experiment. Thereafter, feeds and water were re-introduced 2 h post drug administration.

Sampling and processing: Blood samples were obtained by venupuncture through the left jugular or brachial veins into EDTA tubes at times 0 (pre treatment), 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24-h (post treatment). It was ensured that the differences between the targeted and the actual sampling times were not more than 2 min. The samples were centrifuged at 3000 rpm for 10 min at 37°C and the supernatant (plasma) collected into plastic micro-tubes. These were stored at -4°C and analyzed 24 h following sampling.

Plasma analysis of enrofloxacin: A quantitative-qualitative agar diffusion microbiological assay using blank disks (7 mm) was employed (Kwasi *et al*., 1999; Ehab *et al*., 2008; Andres *et al*., 2009). This assay is based on the concentration–dependent variation of the inhibitory effect of antibiotics on a test bacterium, producing a concentration-response (zone of inhibition) linear relationship. The test organism used was *Escherichia coli*, NCTC10418 cultured on nutrient agar medium (Bryant, 1981; Dowling *et al*., 1995).

The blank disks were adequately saturated with enrofloxacin-spiked blank and treated plasma samples as the case may be. The impregnated disks were then carefully and firmly placed onto the surface of the *E. coli*-seeded nutrient agar (n = 3). This was allowed to diffuse for 5 min after which they were incubated at 37°C in an aerobic condition overnight. Subsequently, the diameters of zones of inhibition were measured with the aid of a transparent rule to the nearest millimeter. Each sample was analyzed in triplicate.

A seven-point standard curve was constructed by spiking blank chicken plasma with concentrations of analytical enrofloxacin salt ranging from 0.02-5.00 µg/ml. A linear curve of plasma enrofloxacin concentrations versus diameters of inhibition zones was obtained (R^2 = 0.89). Plasma concentrations of enrofloxacin were determined by comparing the zone of inhibition diameters with the standard curve. The absence of interfering endogenous compounds was demonstrated in antibacterial-free plasma obtained at time 0 (pretreatment) which showed no visible zone of inhibition around the impregnated disks. The Limit of Quantification (LOQ) was defined visually as the smallest amount of drug that produced a clearly distinguishable zone of inhibition around the edges of enrofloxacin-saturated disks on nutrient agar media and was estimated to be 0.02 µg/ml (Andres *et al*., 2009).

Pharmacokinetics and statistical analyses: Plasma concentrations of enrofloxacin versus time data obtained during the study were utilized for calculating various pharmacokinetic variables using a non-compartmental analysis. The peak concentrations, C_{max} and time to peak, T_{max} were obtained from the plasma concentration-time data directly. The areas under the plasma concentration of enrofloxacin versus time curves from time 0 to the last sample collected (AUC_{0-24}) were calculated using linear trapezoidal method (Gilbadi and Perrier, 1982; Baggot, 2001). While AUC_{0-4} was derived from AUC_{0-24} + AUC_{24-4}, where AUC_{24-4} = $C_{24/ß}$. PK/PD integration for the three enrofloxacin brands was based on C_{max}/MIC_{90} ratio (Baggot, 2001). The value of C_{max}/MIC_{90} ≥10 was considered for accepting the null hypothesis of therapeutic efficacy and prevention of

resistant bacteria strain development in poultry. The upper value of MIC_{90} range, 0.06 µg/ml reported by (Sanjib *et al*., 2005) for most avian pathogenic bacterial organisms was used as the Pharmacodynamic (PD) biomarker.

Statistical analysis on the plasma concentration-time and pharmacokinetics profiles were carried out using two-way Analysis of Variance (ANOVA). Significant difference were determined using Dunett test at $p<0.05$. All data were reported as mean±SEM.

RESULTS

Composite mean plasma concentrations of enrofloxacin at different time points and curves following a single oral administration of the brands at a dose level of 20 mg/kg b.w to chickens are presented in Table 1 and Fig. 1 respectively. The plasma concentrations at the time points sampled for the three brands were significantly different ($p<0.05$).

Table 1: Mean plasma concentrations of enrofloxacin in broiler chickens following a single oral administration of three brands at a dose level of 20 mg/kg b.w

	Mean plasma concentration (µg/ml)		
Post administration Time (h)	Conflox®-vet (Reference)	Kenflox®	Pulmotryl®
0.25	0.38±0.018	0.13±0.023	0.29±0.000
0.50	0.77±0.035	0.61±0.035	0.44±0.025
1.00	1.00±0.055	0.83±0.038	0.48±0.039
2.00	0.86±0.030	0.91±0.024[a]	0.63±0.004
3.00	0.65±0.020	0.84±0.035	0.52±0.046
4.00	0.42±0.027	0.54±0.035[a]	0.41±0.023[a]
6.00	0.19±0.036	0.24±0.038	0.20±0.030[a]
8.00	0.12±0.025	0.17±0.025	0.14±0.025
10.00	0.09±0.000	0.14±0.018	0.12±0.025[a]
12.00	ND	0.11±0.000	0.09±0.001
24.00	ND	ND	ND

Values are mean±SEM (n = 12); [a]Data not significantly different (p>0.05) from the Reference drug; ND-Not detected and NA-Not applicable

The plasma pharmacokinetics parameters are presented in Table 2. Peak plasma concentrations of enrofloxacin (C_{max}), 1.00±0.004, 0.91±0.024 and 0.63±0.004 µg/ml were obtained in animals given conflox®-vet, kenflox® and pulmotryl® brands respectively. The time taken to reach this (T_{max}) in animals administered conflox®-vet was 1 h but 2 h when kenflox® and pulmotryl® brands were administered. The AUC_{0-24} and AUC_{0-4} values for the three formulations were significantly different ($p<0.05$). The highest mean value was observed in animals given conflox®-vet while the least value was obtained in chickens administered brand pulmotryl®.

The PK/PD integrations for the three formulations were calculated and values presented in Table 3. The PK/PD ratios ($Cmax/MIC_{90}$) for conflox®-vet, kenflox® and pulmotryl® brands were 16.67, 15.17 and 10.50 respectively. While the values of the estimated areas under the inhibitory plasma concentration-time curve ($AUIC_{0-4} = AUC_{0-4}/MIC_{90}$) were 72.50, 93.17 and 69.00h for conflox®-vet, kenflox® and pulmotryl® brands respectively. All experimental animals remained in good health during and after the study period.

Table 2: Pharmacokinetics parameters of enrofloxacin obtained after oral administration (20 mg/kg b.w) of different brands in broiler chickens

	Brand		
Pharmaco-kinetics parameter	Conflox®-vet (Reference)	Kenflox®	Pulmotryl®
C_{max} (µg/ml)	1.00±0.055	0.91±0.024[a]	0.63±0.004
T_{max}(h)	1.00±0.000	2.00±0.167	2.00±0.211
K_a (1/h)	1.72±0.030	0.74±0.009	1.42±0.085
$T_{½a}$ (h)	0.40±0.018	0.94±0.026	0.50±0.029
K" (1/h)	0.38±0.010	0.41±0.003	0.32±0.005
$T_{½"}$ (h)	1.82±0.047	1.71±0.010[a]	2.12±0.031
$K_\$$ (1/h)	0.16±0.003	0.08±0.003	0.11±0.002
$T_{½\$}$ (h)	4.33±0.072	7.40±0.009	6.21±0.088
AUC_{0-24} (µg. h/ml)	3.79±0.072	4.90±0.007	4.14±0.034
AUC_{0-4} (µg. h/ml)	4.35±0.072	5.59±0.007	4.14±0.034
$AUMC_{0-4}$ (µg. h^2/ml)	26.88±0.570	39.31±0.072	33.38±1.246
MRT_{oral} (h)	6.02±0.182	7.03±0.072	8.07±0.066
Vd_{area}/F (L/kg)	0.50±0.001	0.88±0.004	0.64±0.003
CL/F (ml/min/kg)	0.08±0.005	0.07±0.006[a]	0.07±0.001[a]

Values are mean±SEM (n = 12), [a]Data not significantly different (p>0.05) from the Reference (Conflox®-vet) drug

Table 3: *In-vivo* PK/PD integration parameters for enrofloxacin after a single oral administration of three brands of 10% enrofloxacin at a dose level of 20 mg/kg b.w

	PK/PD surrogate marker	
Brand	AUC_{0-24}/MIC_{90}(h)	$C_{max}/*MIC_{90}$
Reference	72.50	16.67
X	93.17	15.17
Y	69.00	10.50
Break points	≥100	≥10

*MIC_{90} = 0.008-0.06 µg/ml

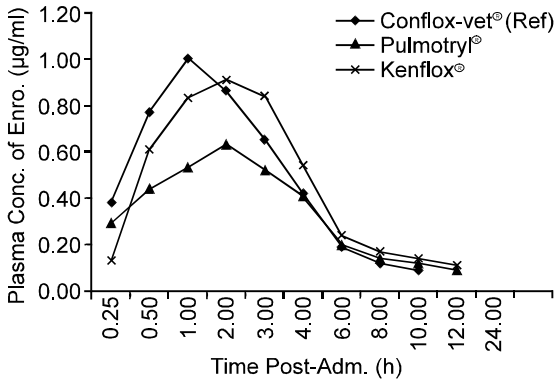

Fig. 1: Plasma concentration-time profile curves of enrofloxacin after oral administration of three brands in broiler chickens at a dose of 20 mg/kg b.w

DISCUSSION

Following administration of a single oral dose of 20 mg/kg b.w, 10% enrofloxacin oral formulations to healthy broiler chickens, therapeutic concentration of the active moiety was attained 15 min post administration in all the animals. The concentration was detected up to 10 h in the plasma of chickens given conflox®-vet brand and up to 12 h in the animals administered kenflox® and pulmotryl® brands. The mean plasma concentrations of enrofloxacin in the three groups were significantly different ($p<0.05$), but the concentrations in all the groups were above the minimum therapeutic concentration reported for enrofloxacin in chickens (0.008-0.06 µg/ml). Differences in the formulations could be responsible for the significant difference.

The mean peak plasma concentrations (C_{max}), 1.00±0.004, 0.91±0.024 and 0.63±0.004 µg/ml obtained in animals given conflox®-vet, kenflox® and pulmotryl® brands respectively were considerably lower than what has been reported in broiler chickens, 2.44±0.06 µg/ml (Anadon *et al*., 1995) at a dose level of 10 mg/kg b.w. But the mean C_{max} in the present experiment is similar to 0.99±0.08 µg/ml (Kwasi *et al*., 1999) and 0.98 µg/ml (Posyniak *et al*., 2007) following oral administration of enrofloxacin at a dose level of 5 mg/kg b.w in broiler chickens. The time taken to reach maximum plasma concentration (T_{max}) in animals administered conflox®-vet and kenflox® brands is similar to 1.68 h (Anadon *et al*., 1995), 2.0 h (Posyniak *et al*., 2007) after a single oral administration at a dose level of 10 mg/kg body weight. These dissimilarities could be due to the differences in the administered doses and possible effects of the recipients in the formulations.

The area under the plasma concentration-time curve (AUC) is a useful index of the biological availability of the drug (extent of absorption). In the present study, the mean AUC_{0-24} and AUC_{0-4} values for the three formulations were significantly different ($p<0.05$). The highest mean value was observed in animals given kenflox®, while the least value was obtained in chickens administered brand pulmotryl®. This indicates that exposure to enrofloxacin is more when the former is administered to chickens at this dose and route. The present values are similar to the value reported by Haritova *et al*. (2004) in chickens. The differences are likely due to the difference in the dosages, routes of administrations and the ingredients used in formulating these brands. Generally, the plasma pharmacokinetics profiles of enrofloxacin following administration of the three brands differed significantly ($p<0.05$).

The clinical effectiveness of Aminoglycosides and Fluoroquinolones is influenced by the height of peak plasma concentration (C_{max}) relative to MIC (C_{max}/MIC) and the area under the plasma concentration-time curve that is above the MIC during the dose interval (AUIC = AUC/MIC). The former is reported to be more significant for Fluoroquinolones where maximum activity is achieved when C_{max} is about 10 fold above the MIC (Baggot, 2001). Based on the above results, all the brands may perhaps be considered effective and will not lead to the emergence of resistant bacterial organisms in chickens when oral dose of 20mg/kg b.w is given to chickens.

Conclusion: Since C_{max}/MIC_{90} ratios obtained following a single oral dose (20 mg/kg b.w) administration of the three brands are above the recommended values, it is likely that this treatment will be effective in chickens infected with common pathogenic bacterial organisms. This also suggests that chances for emergence of resistant bacterial strains following their administrations will be minimal in this animal species.

REFERENCES

Anadon, A., M.R. Martinez-Larranaga, M.J. Diaz and P. Bringas, 1995. Pharmacokinetics and residues of enrofloxacin in chickens. Am. J. Vet. Res., 56: 501-506.

Andres, F.G., A. Maria, A.R. Carlos and V. Omar, 2009. Application of microbiological assay to determine pharmaceutical equivalence of generic intravenous antibiotics. BMC Clin. Pharmacol., 9: 1-11.

Baggot, J.D., 2001. The physiological Basis of veterinary clinical pharmacology. 1st Edn., Blackwell, London.

Bryant, M.C., 1981. The assay of Antibiotics in Body Fluids. In: Laboratory Control of Antibacterial Chemotherapy. 1st Edn., John Wright, London, pp: 102-123.

Craig, W.A., 1993. Pharmacodynamics of antimicrobial gents as a basis for determination of dosage regimens. E. J. Clin. Microbiol. Infect. Dis., 1: 6-8.

Dowling, P.M., R.C. Wilson, J.W. Tyler and S.H. Duran, 1995. Pharmacokinetics of Ciprofloxacin in Ponies. J. Vet. Pharmacol. Therap., 18: 7-12.

Ehab, B., M.G. Saad, M.A. Alaeldein, F.S. Ahmad and M.A. Ahmad, 2008. Pharmacokinetics and Bioequivalence of two norfloxacin oral dosage forms (Vapcotril-10%® and Mycomas 10%®) in healthy broiler chickens. Int. J. Poult. Sci., 7: 289-293.

Gilbadi, M. and P. Perrier, 1982. Pharmacokinetics. 2nd Edn., Marcel Dekker, Inc, New York.

Haritova, A., H. Djeneva, L. Lashev, P. Sotirova, B. Gyurov and M. Stefanova, 2004. Pharmacokinetics and PK/PD modeling of enrofloxacin in *Meleagris Gallopavo* and gallus domesticus. Bulg. J. Vet. Med., 7: 139-148.

Kwasi, B., W.D. Black and M.E. Scott, 1999. Pharmacokinetics of enrofloxacin given by oral, intravenous and intramuscular routes in broiler chickens. Canad. J. Vet. Res., 63: 193-200.

Lakshmi, K., 2006. Modeling success in PK/PD testing, drug discovery and development.

Marie, G., 2007. PK/PD Data analysis 1-basic concept. Med. Prod. Agency.

Meinen, J.B., J.T. McClure and E. Rosin, 1995. Pharmacokinetic of enrofloxacin in clinically normal dogs and mice drug pharmacodynamics in neutropenic mice with *E. coli* and *Staphylococcal* infections. Am. J. Vet. Res., 38: 732-749.

Mouton, J.W. and P.M. Tulkens, 2005. PK/PD modeling: Clinical implication. ICAAC–ISAP PK/PD workshop.

Posyniak, A., J. Zmudzki, J. Niedzielska and B. Biernacki, 2007. Bioequivalence study of two formulations of enrofloxacin following oral administration in chickens. Bul. Vet. Res. Inst. Pulawy, 45: 353-358.

Randandt, K.M., M.C. Randall and M.N. Dudley, 1992. Interactions of fluoroquinolones with other drugs: Mechanisms, variability, clinical significance and management. Clin. Infect. Dis., 14: 272-284.

Reiko, S., T. Yusuke, K. Mitsuo, A. Naoki and S. Kihachiro, 2006. Pharmacokinetics-Pharmaco-dynamics relationship of arbekacin for treatment of patients infected with methicillin-resistant *Staphylococcus aureus.* J. Antimicrob. Agent Chemother., 50: 3768-3769.

Sanjib, I., C.B. Chandana, M. Pritam and B. Mohan, 2005. Pharmacokinetics studies of enrofloxacin in Yak after intramuscular administration. Ir. J. Pharmacol. Therap., 4: 91-94.

Sumano, L.H. and O.L. Gutierrez, 2000. Problematica del uso de la enrofloxacin en la aviculture en Mexico. Vet. Mex., 2: 137-145.

Sumano, L.H. and O.L. Gutierrez, 2001. Strategic administration of enrofloxacin in poultry to achieve higher serum concentrations. In: Proceedings of the Fiftieth Western Poultry Disease Conference. University of California, Davis, pp: 45-48.