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

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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 for most pathogenic bacterial organisms in poultry in all the groups. The PK/PD integration ($C_{\text{max}}$/$\text{MIC}_{\text{90}}$) values, 16.67, 15.17 and 10.5 h were obtained in animals administered confloxx®-vet, kenfloxx® 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, $C_{\text{max}}$ 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, $C_{\text{max}}$/MIC; the ratio of 24-h area under the plasma concentration-time curve to minimum inhibition concentration, $\text{AUC}_{\text{24-h}}$/MIC and the duration of time that plasma levels exceed the minimum inhibition concentration, $T >\text{MIC}$ (Baggot, 2001; Marie, 2007). Clinical response usually correlates with $\text{AUC}_{\text{24-h}}$/MIC and $C_{\text{max}}$/MIC for concentration-dependent antimicrobial agents, but the latter, $C_{\text{max}}$/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 $C_{\text{max}}$/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...
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\text{max}/MIC using MIC\textsubscript{90} against the most common pathogenic bacterial organisms in poultry (Sanjib et al., 2005).

**MATERIALS AND METHODS**

**Study products:** Conflox\textsuperscript{®}-vet (10% enrofloxacin) from India (Batch No: 70002, Exp. 06-2012); kenfloxE (10% enrofloxacin) from Holland (Batch No: 0811703, Exp. 03-2011) and pulmotryl\textsuperscript{®} (10% enrofloxacin and 1% bromhexine hydrochloride) from Jordan (Batch No. 08-022, Exp. 06-2012). Pure enrofloxacin (\textgeq98%) from Sigma- Aldriech, USA was used as a standard. Nutrient agar by Lab M, USA and Escherichia coli, NCTC10418 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. Plasma concentrations of enrofloxacin versus time data obtained from AUC\textsubscript{0-24}, AUC\textsubscript{0-4}, AUC\textsubscript{24/ß}, PK/PD integration for the three enrofloxacin brands was based on C\textsubscript{max}/MIC\textsubscript{90} ratio (Baggot, 2001). The value of C\textsubscript{max}/MIC\textsubscript{90} \textgeq10 was considered for accepting the null hypothesis of therapeutic efficacy and prevention of

**Sampling and processing:** Blood samples were obtained by venupuncture through the left jugular or brachial veins into EDTA tubes at times 0 (pretreatment), 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.
The plasma pharmacokinetics parameters are presented in Table 2. Peak plasma concentrations of enrofloxacin (C_{\text{max}}), 1.00±0.004, 0.91±0.024 and 0.63±0.004 µg/ml were obtained in animals given confolex®-vet, kenfloxa and pulmotryl® brands respectively. The time taken to reach this (T_{\text{max}}) in animals administered confolex®-vet was 1 h but 2 h when kenfloxa and pulmotryl® brands were administered. The AUC_{0-24} and AUC_{0-10} values for the three formulations were significantly different (p<0.05). The highest mean value was observed in animals given confolex®-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 (C_{\text{max}}/MIC_{90}) for confolex®-vet, kenfloxa 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 (AUC_{0-24}/MIC_{90}) were 72.50, 93.17 and 69.00h for confolex®-vet, kenfloxa and pulmotryl® brands respectively. All experimental animals remained in good health during and after the study period.
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 confolex®-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 confolex®-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 confolex®-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 (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