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Influence of oral co-administration of a preparation containing calcium and magnesium and food on enrofloxacin pharmacokinetics

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ABSTRACT

The objective of this study has been to determine the influence of food and ions on the pharmacokinetics of enrofloxacin (ENRO) in turkeys, administered *per os* at a dose of 10 mg/kg of body weight (b.w.). Co-administration of ENRO with ions or with food significantly retarded its absorption, and the interaction was more pronounced when the drug was given together with food. The bioavailability of ENRO was 65.78 ± 7.81% and 47.99 ± 9.48% with ions and food, respectively. The maximum concentration (C_{max}) in plasma of animals exposed to ions reached 0.87 ± 0.26 μg/ml in a t_{max} of 2.07 ± 0.76 h; in animals which were fed while medicated, the analogous parameters were 0.36 ± 0.13 μg/ml and 8.06 ± 3.08 h. The PK/PD analysis demonstrated that a decrease in the concentration of ENRO in turkeys' blood due to the interaction with ions or food might impair the drug's clinical efficacy toward some pathogenic microorganisms in turkeys if a routine dose of 10 mg ENRO/kg b.w. is administered.

1. Introduction

Enrofloxacin (1-cyclopropyl-7-(4-ethylpiperazin-1-yl)-6-fluoro-4-oxoquinoline-3-carboxylic acid; ENRO) is a synthetic, chemotherapeutic anti-bacterial drug which belongs to fluoroquinolones (FQ). It is popular in veterinary medicine mainly because of its good pharmacokinetic properties, low toxicity and a broad range of microbial effects, including Gram-positive and Gram-negative aerobic bacteria as well as atypical pathogens such as *Mycoplasma* and *Chlamydophila* (Brown, 1996). The mechanism through which the drug acts involves topoisomerase inhibition, which leads to disorders in the replication of the genetic material of microorganisms (Brown, 1996).

Quinolones are distinguished by their capability of forming complexes with polyvalent metal cations, such as Ca^{2+}, Mg^{2+}, Fe^{2+} and Al^{3+}. The mechanism of this phenomenon has not been thoroughly investigated, although it is attributed to the binding of ions with the 3-carboxyl-4-oxo group in a molecule of FQ (Lomaestro and Bailie, 1995).

The interaction of quinolones with polyvalent cations in the digestive tract can disturb the absorption of these drugs and consequently lead to unwanted changes in their pharmacokinetics, which means that their efficacy is adversely affected. The decreased bioavailability of FQ coinciding with the exposure of the digestive tract to high levels of polyvalent cations has been observed in experiments on humans (Lazzaroni et al., 1993; Lober et al., 1999; Nix et al., 1990; Polk et al., 1989), dogs (Wallis et al., 1996) and broiler chickens (Aguilera et al., 2007; Sumano et al., 2004). Also, the presence of food affects the absorption of ENRO from the digestive tract, as has been demonstrated in experiments on humans (Frost et al., 1989; Ledergerber et al., 1985; Somogyi et al., 1987), pigs (Nielsen and Gyrd-Hansen, 1997), and horses (Steinman et al., 2006). The presence of food has an undesirable influence on the pharmacokinetics of FQ, which may impair the efficacy of treatment with these drugs.

Based on the results of up-to-date research, it can be claimed with a high degree of probability that the current guidelines for the pharmacotherapy of bacterial diseases in poultry with the application of FQ might need some improvement, as they do not account for losses in the absorption of these medications from the digestive system due to their binding to bivalent ions found in water and feeds. As a result, the bioavailability of a given drug can be diminished, its absorption from the gastrointestinal tract (GIT) may take longer or its maximum concentration in blood plasma might be lowered. These effects are associated with decreased efficacy and reductions in the incidence of

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clinical success; another consequence could be the increased drug-resistance among microorganisms. Consequently, the treatment may have to last longer, to the detriment of the efficiency of production.

Considering the unrecognized question of concurrent administration of food and ENRO on the pharmacokinetics of the drug in birds, and in view of the fact that mineral supplements are widely used in poultry rearing, a study has been undertaken to clarify the effect of the aforementioned factors on the pharmacokinetics of ENRO in turkeys, paying special attention to the process of absorption of the drug from the GIT.

2. Materials and methods

2.1. Animals and drugs

Thirty-two, four-week old (male and female), healthy broiler turkeys (type BIG-6) were obtained from a commercial farm in Kieźliny near Olsztyn and transported to a vivarium of the Faculty of Veterinary Medicine, University of Warmia and Mazury. The vivarium was maintained at 22 °C and at a relative humidity of 60–65%. The birds were observed for one week and fed a standard turkey grower diet (crude protein 25%, crude fiber 4%, calcium 1%, phosphorus 0.8%, sodium 0.16%, lysine 1.65%, methionine 0.65%); feed and water were provided on an *ad libitum* basis. No clinical signs of disease were noticed. Before the experiment, the birds had not been medicated with any drugs.

For intravenous administration (i.v.), Enrobioflox 5% veterinary solution for injections (Vetoquinol Biowet, Gorzów Wielkopolski, Poland) was given into the right brachial vein. For oral administration (p.o.), Enrofloxan 10% veterinary oral solution (Biofaktor, Skierniewice, Poland) was dissolved in *natrium chloratum* (Biofaktor, Skierniewice, Poland) and given via a gastric tube as gavage. ENRO was administered in all the trials at a nominal dose of 10 mg/kg of body weight (b.w.).

2.2. Experimental design

The animals were divided into four groups of eight birds each. Before the experiment, feed was withheld for 8 hours and water was not given for one hour. In the first group (control), feed was made available 3 hours after the administration of the drug; in the second group (experimental group no 1), the drug was administered first, then a mineral preparation containing (calcium chloride 140 g/L, magnesium chloride 3.2 g/L and cobalt chloride 25 mg/L as adjunct, Neolait, France) was given and finally feeding was resumed 3 h later; in the third group (experimental group no 2), feeding was resumed 30 minutes prior to the administration of the drug. The fourth group, needed for calculating the bioavailability of ENRO, consisted of birds receiving the drug intravenously (the i.v. group). In all the groups, access to water (in the i.v. group also feed) was made possible 1 h after the administration of the drug (in experimental group no. 1 the water with mineral preparation). For 0.5 h after the administration of ENRO, the birds were submitted to observation in order to exclude regurgitation. The study had been registered and approved by the Local Ethics Commission (Ethic Commission Opinion No. 14/2006).

Blood samples (1.0 ml each) were collected into heparinized tubes from the left brachial vein with a venflone cannula (HMD Healthcare Ltd., UK) at 0, (0.083 i.v. group), (0.25 i.v. group) 0.5, (0.75 i.v. group), 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0 and 24.0 h after drug administration. Plasma was separated by centrifugation at 1650 × g for 10 min. at 4 °C and was stored at –32 °C until assay.

2.3. Drug analysis

2.3.1. Chemicals and reagents

ENRO (93106-60-6) and anthranilic acid (118-92-3) as an internal standard (IS) were purchased from Fluka (Milwaukee, WI, USA). Ammonium hydroxide, ammonium acetate, acetonitrile, methanol, 1,2-dichloroethane, 85% phosphoric acid, water all gradient grade were supplied by J.T. Baker (Phillipsburg, NJ, USA). Prepared samples before high performance liquid chromatography (HPLC) analysis were filtered through Nylon, Syringe Filter 0.45 μm, 13 mm diameter (SMI-LabHut, Gloucester, UK).

2.3.2. Chromatography

The plasma concentration of ENRO was determined using the sample preparation and the HPLC analytical method described previously by Jakubowski et al. (2010), with minor modifications. The modifications were as follows: lack of pre-column; using 25 μL of ENRO and IS instead of 50 μL while preparing the calibration curves and samples for analyses; extension calibration concentration range from 0.075 to 10.0 μg/ml; using 4 quality control points (QC) instead of 3. The method comprises liquid–liquid extraction with 1,2-dichloroethane and was performed on Agilent 1100 series HPLC system (Agilent Technologies, Inc., Santa Clara, California, USA) with fluorescent detection (the excitation wavelength of 300 nm and the emission wavelength of 448 nm) and RP C18 column (150 × 3 mm, 3 μm particle size, Phenomenex, Torrance, CA, USA) using acetonitrile-ammonium acetate buffer in gradient elution. The current analytical method was fully validated in our laboratory because of some modifications in analytical procedure according to the United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) bioanalytical method validation requirements (FDA and CDER, 2000, 2001; EMEA, 2011).

2.3.3. Method validation

During the validation procedure, as described above, the limit of detection (LOD) was set at 0.05 μg/ml (signal to noise ratio was not lower than 3:1) and the lowest limit of quantitation (LLOQ) equaled 0.075 μg/ml (signal to noise ratio was not lower than 10:1). Plasma free from ENRO and IS, obtained from blood drawn from clinically healthy turkeys, was used for preparing a calibration curve. The curve included 9 points within the range of concentrations from 0.075 to 10.0 μg/ml, of which 4 points served for QC: low quality control (LQC), intermediate quality control (IQC), medium quality control (MQC), high quality control (HQC).

The ENRO determination method was characterized by high linearity. The coefficient of correlation r^2 was above 0.99 for all the calibration curves. Differences between particular control points were 2.0–9.0% for accuracy, and the coefficient of variation for precision was within 3.0–8.0% for individual points. The specificity of the method relied on an analysis of six samples of plasma free from ENRO and IS, collected from turkeys for which no significant peaks at the retention time of ENRO were demonstrated. In the cited method, the total recovery was over 90% for both ENRO and IS. ENRO turned out to be stable in an autosampler at 22 °C (a decrease in the concentration did not exceed 7% after 72 h) and during the cycle of thawing and freezing (a decline in the concentration was no higher than 5%) or as the prepared working standard stored in a refrigerator (4 °C) for 7 consecutive days (a decrease in the concentration did not exceed 5%). The matrix, which was plasma, did not demonstrate any response to the signal from the detector, the fact that was verified through analyzing the signal of the matrix with and without the ENRO standard, or while comparing the signal of the same concentration of ENRO in water and in plasma as the matrix.