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Pharmacokinetic and tissue distribution of doxycycline in broiler chickens pretreated with either: Diclazuril or halofuginone

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ABSTRACT

Following IV injection of doxycycline in a dose of 20 mg kg^{-1} b.wt., its serum concentration was best fitted in two-compartment open model in chickens fed either on control or on anticoccidials-containing rations. Diclazuril and halofuginone resulted in a significant short distribution half-life ($t_{½\alpha}$) (7.17 ± 0.39 and 11.88 ± 1.05 min, respectively) and increased total body clearance (Cl_{tot}) 0.37 ± 0.024 and 0.295 ± 0.034 L/kg/h, respectively. Following oral dosing the tested drug absorbed with $t_{½ab}$ of 41.38 ± 1.6, 17.48 ± 0.86 and 41.83 ± 1.8 min, respectively and their C_{max} values (3.18 ± 0.18, 5.425 ± 0.48 and 0.986 ± 0.037 μg/ml) were attained at 2.07 ± 0.097, 1.403 ± 0.074 and 2.55 ± 0.106 h. For doxycycline alone and in presence of diclazuril and halofuginone, respectively. Systemic bioavailability was 22.64 ± 3.46, 86.74 ± 9.23 and 22.38 ± 3.09%, respectively. Following IM injection $t_{½ab}$ were 9.096 ± 1.34 for doxycycline alone, 16.24 ± 2.21 and 15.6 ± 1.7 min in the presence of diclazuril and halofuginone, respectively. C_{max} was 3.10 ± 0.28, 4.63 ± 0.57 and 0.55 ± 0.07 μg/ml reached at 0.8 ± 0.083, 1.13 ± 0.126 and 1.21 ± 0.105 h. For the antibiotic alone, and in presence of either diclazuril and halofuginone, respectively. Systemic bioavailability was 22.41 ± 3.86, 88.97 ± 12.9 and 12.31 ± 0.99% in chickens fed on anticoccidial-free, diclazuril- and halofuginone-containing rations, respectively. Both the tested anticoccidials induced higher doxycycline tissue residues in all tested tissue samples.

1. Introduction

Medication in poultry farms usually depends on supplementing feeds with antimicrobial, antifungal, antimycotoxine, growth promotant and anticoccidial drugs. More than 15 compounds of anticoccidial drugs are approved for use in poultry farms (Jones and Ricke, 2003). Diclazuril is a benzeneacetonitrile anticoccidial used in concentration of 1 ppm in poultry ration (Lindsay et al., 1995) and halofuginone is a member of quinazoline group that acts as coccidiostat at concentration of 1.5 ppm and as coccidiocidal at 3 ppm in the ration (Brander et al., 1991). Doxycycline is a derivative of tetracycline (α-6-deoxytetracycline) often used in treatment of various diseases in poultry (Croubels et al., 1998). It is active against Gram + ve, Gram −ve, aerobic and anaerobic bacteria, also affecting Mycoplasma, spirochetes, Rikettsia, chlamydia and anaplasma species (Laczay et al., 2001; Prats et al., 2005). Continuous addition of small amounts of anticoccidial drugs in the daily ration in intensive poultry farms as prophylaxis, may affect the kinetic behaviour as well as the efficacy of the antibacterial drugs used for treatment of bacterial diseases.

The present study aims to elucidate the effect of diclazuril and halfuginone as common anticoccidials on the disposition kinetic of doxycycline in broiler chickens during their concomitant administration.

2. Material and methods

2.1. Drugs

Doxycycline: It was obtained from Pharmaswede Co., Cairo, Egypt as a doxycycline HCl [each 100 gm contains 98.4 g doxycycline base].

Diclazuril (Clinacox®): It is supplied by Pharmaceutic Animal Health Division Pfizer. It is formulated as a premix 0.5%.

Halofuginone hydrobromide (Stenorol®): Supplied by Hoechst-Russel Vet. Co., as powdered form containing 0.5 g halofuginone per kg powder.

2.2. Birds

Sixty-five healthy broiler (Hubard breed) chickens of both sex with an average body weight of 1500–1850 g and of 45–50 day old age were used. They were housed in cages and supplied with water ad libitum. Chickens were fed on a balanced ration free from any anticoccidial and antibiotic drugs for 15 days before

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starting experiments to ensure complete clearance of their bodies from any drug residues. This study was performed in accordance with all local regulations of the ethical committee for research and animal experiments.

2.3. Experimental design

2.3.1. Single dose studies (pharmacokinetic studies)

Three main groups of 10 chickens each were used. Birds of the 1st group were fed anticoccidial-free ration allover the study. Those in the 2nd and 3rd groups were fed a ration containing diclazuril (1 mg kg^{-1} feed) and halofuginone (3 mg/kg feed) for five consecutive days, respectively. At the end of 5th day of feeding, doxycycline was injected i.v. in a single dose (20 mg/kg b.wt.) in the right brachial wing vein of each bird of these three main groups.

Blood samples were taken from the left brachial wing vein of each chicken just before and at 5, 10, 15, 30 min and 1, 2, 4, 6, 8 and 10 h post antibiotic injection. Blood samples were allowed to clot at room temperature, centrifuged at 3000*g* for 10 min and serum were collected and stored at −20 °C until assayed for doxcycycline.

Chickens were kept for 15 days devoid of any antibiotic or anticoccidial drugs to ensure complete withdrawal of the administered drugs. Each of the 3 main groups was subdivided into 2 subgroups of 5 chickens each. Chickens in the 2nd and 3rd groups were fed on diclazuril and halofuginone containing ration, respectively, for five consecutive days, while those of the 1st group were kept free of anticoccidial therapy. At the 5th day of feeding, doxycycline was administered in a single oral dose (20 mg/kg b.wt.) to all the chickens of the 1st subgroups, whereas those of the 2nd subgroups received doxycycline intramuscularly (thigh muscle) in the same dose.

Blood samples were collected from each bird in all groups at the same time intervals as mentioned before. Serum samples were collected and stored at −20 °C until assayed for doxycycline.

2.3.2. Multiple dose study

Three groups of 30 birds each were used in this study. Chickens of the 1st group were fed an anticoccidial-free ration allover the time of experiment. Those in the 2nd and 3rd groups were fed on diclazuril (1 mg/kg feed) and halofuginone (3 mg/kg feed) containing rations, respectively, allover the experiment. At the end of 5th day of feeding, each of the 3 groups were subdivided into 2 equal subgroups of 15 chickens each. Doxycycline was administered orally to birds of the three 1st subgroups, whereas it is injected intramuscularly in those of the three 2nd subgroups. This is continued once daily for five consecutive days in all groups. Blood samples were collected every 24 h for five successive days from the wing veins of five marked chickens in each subgroup. Serum was separated and stored at −20 °C until assayed for doxycycline. From each subgroup 3 chickens were slaughtered every 48 h after the last dose of doxycycline administration.

Tissue samples including liver, spleen, lung, kidney, heart, intestine, breast and thigh muscles were collected from each slaughtered chicken, stored at −20 °C until doxycycline assay.

2.3.3. Analytical procedure

Doxycycline concentration in serum and tissue samples was determined by the microbiological assay method using *Bacillus cereus var. mycoides* as a test organism (Bernard et al., 1971). The serum concentrations of doxycycline were measured by bioassay using an agar-gel diffusion method employing *Bacillus cereus vat mycoides* ATCC 11778 as the test organism. Standard solutions were prepared in antibiotic-free chicken's serum in appropriate serial dilutions. The experimental samples and standards were analysed in triplicate. The measured widths of the zones of inhibition were converted to concentrations using standard curves developed for each plate. Standard curves were prepared as described by Dornbush and Abbey (1972). The calibration graphs obtained for doxycycline in serum were linear within the range 0.05–0.80 μg/ml; Y = 14.2 + 17.5×. The average recovery was 105%, the overall precision (between days, reported as relative standard deviation) was 23%, and the limit of detection for the assay for serum samples was 0.01 μg ml^{-1} and the quantitation limit was 0.025 μl ml^{-1} or g^{-1}.

Standard curve for doxycycline was constructed using antibiotic-free pooled serum samples collected by the slaughtering of five non-medicated chickens. The *in vitro* protein-binding percent of the tested antibiotic was determined by the method of Craig and Suh (1980) with concentrations of 0.156, 0.125, 0.1, 0.08 and 0.064 μg/ml.

2.3.4. Pharmacokinetic analysis

A computerized curve-stripping software program (Rstrip, Micromath Scientific software, Salt lake city, UT, USA) was used for the determination of the best-fit compartmental model and for the estimation of the model-dependent pharmacokinetic parameters. Following i.v. injection, doxycycline serum concentration time data for each chicken were fitted in a two-compartment open model according to the following equation:

$$Cp = Ae^{-\alpha t} + Be^{-\beta t}$$

where Cp = the serum drug concentration at time *t*. A and B = the intercepts of the distribution and elimination lines with the concentration axis, respectively, they were expressed in μg ml^{-1} α and β are the distribution and elimination rate constants, respectively, expressed in units of reciprocal time (h). e is the base of natural logarithm. The distribution and elimination half-lives ($t_{½\alpha}$ and $t_{½\beta}$), the rate constants for drug transferring from central compartment to peripheral one (K_{12}) and from tissues to central compartment again (K_{21}), the volume of distribution was calculated by the area (Vd_{area}) and B (Vd_B) methods. The total body clearance (Cl_B) was calculated according to standard equations of Baggot (1978) and Gibalidi and Perrier (1982).

Following oral and I.M. administrations, data were analysed by compartmental and non-compartmental methods based on the statistical moment theory (Yamaoka et al., 1978). The peak plasma concentration (C_{max}) and the time needed to reach this concentration (T_{max}) were calculated.

K_a is the absorption rate constant (h^{-1}) and K_{el} is the elimination rate constant (h^{-1}). $AUC_{o-infinity}$ is the area under the serum concentration-time curve from zero to infinity by the trapezoidal rule.

C_{max}: Maximum serum concentration of drug in blood after parenteral administration (μg/ml).

T_{max}: The time at which the maximum concentration of the drug was reached after parentral administration (h).

The systemic bioavailability (F) = (AUC oral or i.m./$AUC_{I.V.}$) × 100.

2.4. Statistical analysis

The obtained results are represented as mean ± standard error (S.E.). The pharmacokinetic parameters in the presence and absence of diclazuril and halofuginone were statistically analyzed using students *t*-test (Snedecor and Cochran, 1980).

3. Results

The semilogarithmic serum concentration–time curves of doxycycline following IV (Fig. 1), oral (Fig. 2) and IM (Fig. 3), routes in a single dose (20 mg/kg b.wt.), showed lower serum doxycycline concentration in diclazuril and halofuginone containing ration, than in anticoccidial-free ration. Following IV injection the drug serum concentration declined in a biphasic pattern that can be described by a two-compartment open model. The kinetic parameters obtained after IV injection are shown in Table 1. The obtained results showed significant higher K_{12}/K_{21}, Vd_B, Vd_{area}, Cl_{tot} and K_{el} as well as different tissues doxycycline concentrations in chickens taken diclazuril and halofuginone containing ration than in anticoccidial-free ration (Fig. 1 and Table 1).

The semilogarithmic serum concentration–time curves of doxycycline following oral and IM administrations in a single dose (20 mg/kg b.wt.) in chickens fed with control ration, diclazuril- and halofuginone-containing ones are represented in Figs. 2 and 3 and kinetic parameters of the tested drug following oral and IM administration are shown in Tables 2 and 3, respectively.

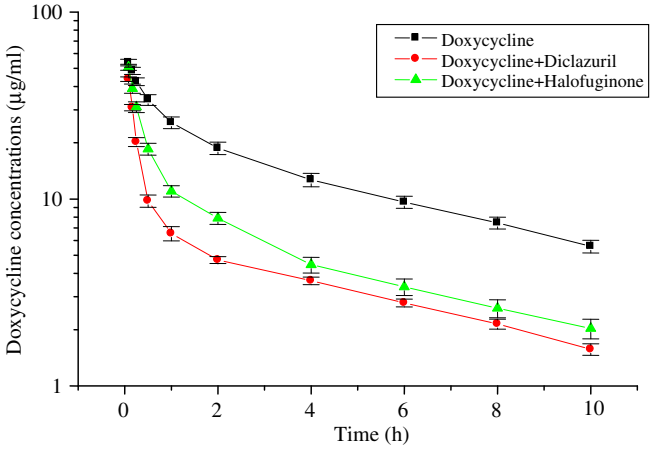

Fig. 1. Mean serum concentration of doxycycline(μg/ml)following a single intravenous administration (20 mg/kg b.wt.) in chickens fed anticoccidial free, diclazuril (1 ppm) and halofuginone (3 ppm) containing rations (Mean ± S.E.) *n* = 5.