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Pharmacokinetics and Bioequivalence of Florfenicol Oral Solution Formulations (Flonicol® and Veterin®10%) in Broiler Chickens

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Abstract

A pharmacokinetic and bioequivalence evaluation of two florfenicol oral solutions was carried out in 28 healthy broiler chickens after oral administration of a single dose of 20 mg/kg bw, according to a randomized, parallel experimental design. The two formulations were: Flonicol® (Mobedco, Jordan) as a test product and Veterin®10% (Centrovet, Chile) as a reference product. The pharmacokinetic analysis was performed using non-compartmental analysis based on statistical moment theory with the help of a commercially available software program (WinNonlin®, Pharsight Corp., Cary, NC, USA). There were no significant differences in the C_{max} (9.02 ± 0.68, 9.20 ± 0.77 µg/ml), t_{max} (1.02 ± 0.13, 1.05 ± 0.30 h), $t_{1/2\beta}$ (1.41 ± 0.06, 1.35 ± 0.05 h), AUC_{0-12h} (26.45 ± 1.33, 26.06 ± 1.20 µg.h/ml), $AUC_{0-\infty}$ (26.61 ± 1.33, 26.26 ± 1.21µg.h/ml), AUMC (71.78 ± 4.65, 69.98 ± 8.80µg.h^2/ml), MRT (2.72 ± 0.18, 2.62 ± 0.27 h), Cl_B/F (12.82 ± 0.63, 12.96 ± 0.60 ml/min/kg) and Vd_z/F (1.55 ± 0.08, 1.51 ± 0.08 l/kg) between Flonicol® and Veterin®10%, respectively. The 90% confidence interval for test: reference ratio of the AUC_{0-12h} (91.86-111.67 µg.h/ml), $AUC_{0-\infty}$ (91.77-111.57 µg.h/ml) and C_{max} (82.36-118.54 µg/ml) were within the European Agency for Evaluation of Medicinal Product (EMEA) and the US Food and Drug Administration (FDA) bioequivalence acceptable range (80%–125%). In conclusion, Flonicol® was found to be bioequivalent to Veterin®10% and can be used as interchangeable therapeutic agents in veterinary practice.

Keywords: Pharmacokinetics; Bioequivalence; Florfenicol; Chicken; High-pressure liquid chromatography

Abbreviations: HPLC – High Performance Liquid Chromatography; LOQ – Limit of Quantification; LOD – Limit of Detection; AUC – Area under the time-concentration curve; AUMC – Area under the first moment curve; MRT – Mean Residence Time; Vd_z/F – Apparent volume of distribution corrected for unknown bioavailability; k_{el} – Elimination rate constant; $t_{1/2\beta}$ - Elimination half-life; Cl_B/F – Total body clearance corrected for unknown bioavailability; C_{max} – Maximum measured plasma drug concentration is measured; t_{max} – Time at which maximum plasma drug concentration; MIC – Minimum Inhibitory Concentration; ANOVA – Analysis of Variance

Introduction

Florfenicol is a broad-spectrum bacteriostatic antibacterial that belongs to amphenicol family, with a wide range of activity against different types of Gram-negative and Gram-positive organisms including: *Mannheimia haemolytica*, *Pasteurella multocida*, *Haemophilus somnus*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Shigella dysenteriae* and *Staphylococcus aureus* [1-3]. In addition, florfenicol is active at lower concentrations than its structural analogs, thiamphenicol and chloramphenicol, against a number of bacterial pathogens and against many chloramphenicol or thiamphenicol-resistant strains [4,5]. Florfenicol is approved in the European Union for use in cattle, sheep, pigs and chickens [6,7].

The efficacy of florfenicol has been demonstrated against many diseases of domestic animals [8-14]. However, to date, studies on the efficacy of florfenicol using pharmacokinetic/ pharmacodynamic (PK/PD) approaches have not been carried out in poultry. Nevertheless, the pharmacokinetics and bioavailability of florfenicol have been investigated in broiler chickens [15-17], turkeys [7] and ducks [18]. Most of these studies used the same original preparation of florfenicol. There is therefore little information available regarding the differences between formulations of florfenicol used in poultry.

Due to it is advantages related to safety and efficacy over thiamphenicol and chloramphenicol, many florfenicol commercial preparations has been approved and employed in several countries for the treatment of serious gastrointestinal and respiratory bacterial infections in poultry and other farm animals [7,11,17]. In order to optimize the clinical outcome and to minimize the development of bacterial resistance, the copied pharmaceutical preparations must be bioequivalent to the innovator product [19,20]. These issues have become an on-going subject of concern within the European Community and the United States of America for registering new and generic drug products [19,21-23].

The aim of the present study was to evaluate the bioequivalence and comparative disposition kinetics of florfenicol following administration as one of two oral solutions; Flonicol® (Mobedco, Jordan) as a test product and Veterin®10% (Centrovet, Chile) as a reference product.

Materials and Methods

Drugs

Test product: Flonicol®—Florfenicol, 10% oral solution, The

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Arab Pesticides & Veterinary Drugs Mfg.Co. (Mobedco), Al-Hassan Industrial Estate, Irbid, Jordan.

Reference product: Veterin®10%— Florfenicol 10% oral solution, Centrovet Ltda., Los Cerrillos 602, Cerrillos, Santiago, Chile.

Animals

Twenty eight chickens (Hubbard x Hubbard), 35-50 days old and weighing 1.8-2.2 kg were used in the study. The chickens were purchased from a local poultry farm. They were placed in the Animal House Facility at Jordan University of Science and Technology. The animals were monitored for 2 weeks for any apparent clinical signs of disease before drug administration. The animal house temperature was maintained at 25 ± 2°C and humidity at 45–65%. The chickens had free access to water and antibacterial-free food ad libitum daily.

Experimental design

The chickens were divided into 2 equal groups of 14 birds each. Chickens of group 1 and 2 were given a single oral dose of Flonicol® and Veterin®10% at a dose level of 20 mg/kg bw, respectively. The dose was chosen according to the manufacturers' instructions. Chickens were weighed prior to drug administration and the doses were calculated accordingly. Florfenicol was given directly into the crop using a thin plastic tube attached to a syringe. Food was withheld for 12h before and 6h after drug administration. Water was provided ad libitum. The study followed a randomized, parallel design.

Samples collection

Blood samples (1-1.5 ml) were collected from the left brachial vein or cutaneous ulnar veins into heparinized tubes at 0 (before treatment), 10, 20, 30, 45 min, and at 1, 1.5, 2, 4, 6, 8, 10, 12 and 24h after drug administration. All blood samples were centrifuged directly at 1000x g for 5 min and plasma was harvested and stored at −20°C until analysis.

Analytical method

Plasma concentrations of florfenicol were determined using a high performance liquid chromatographic (HPLC) method. The HPLC and extraction procedures were modified from previously published methods [24,25]. The HPLC system consisted of a pump (LC-10A DVP) with UV-vis detector (SPD-10 AVP), auto injector (SIL-10A DVP), solvent degasser (DGV-12 A) and Shimadzu class-VP software (Ver 6.12 SP4) (Shimadzu, Japan). Chromatographic separation was performed using a Purospher Star RP-18e (5 µm, 125 mm ×4.6 mm) column (Merck, Germany) with an isocratic mobile phase (25%, acetonitril:water). The mobile phase was filtered through a 0.45 µm membrane filter and degassed. The flow rate was set at 2.0 mL/min and the UV detector was set at a wavelength of 223 nm.

Sample preparation

Plasma samples were separately extracted and chloramphenicol was used as an internal standard in the analytical method. Briefly, frozen plasma samples were thawed at room temperature and then 250 µl plasma was added to Eppendorf tubes containing 50 µl of chloramphenicol (100 µg/ml) (as internal standard). After mixing each sample for 10 seconds, 250 µl of acetone was added and the tubes were then shaken for 30 seconds and centrifuged for 5 min at 1000xg. The clear supernatant was transferred into eppendorf tubes. 1 ml of ethyl acetate was added to the tubes then the mixture vortexed for 30 seconds and centrifuged for 5 min at 1000x g. 1 ml of super layer was transferred into clean glass tubes. The organic layer (ethyl acetate) was harvested and dried by heating at 60°C for 15 min. After evaporation, each residue was reconstituted in 250 µl of mobile phase and 100 µl was injected into the HPLC system.

Calibration curve

A standard calibration curve was prepared by adding 20 µl of florfenicol (1 mg/ml) to 980 µl antibacterial-free chicken plasma. This was further diluted into antibacterial-free chicken plasma to produce standard of 0.05, 0.1, 0.5, 1, 2, 5, 10 and 20 µg/ml. Standard solutions were extracted and analyzed using the same procedure as the unknown samples. Calibration curves were obtained by calculating the ratios of the area of florfenicol to that of chloramphenicol and plotting them against the corresponding concentration of florfenicol spiked in chicken plasma. The areas under the peaks were determined by integration using the software program Class-vp (Shimadzu, Japan).

Validation procedure

A complete validation of the analytical procedure that used for extraction and quantification of florfenicol was performed before analysis of experimental samples from the bioequivalence trials. Linearity, accuracy, precision, recovery and sensitivity were assessed. Two standard calibration curves with 7 concentrations (0.025, 0.05, 0.1, 0.5, 1, 2, 5, 10 and 20 µg/ml) and 6 sets of quality control samples (0.3, 3 and 7 µg/ml) were prepared and analyzed daily for 3 consecutive days. The standard curves were linear over the range of 0.05 – 20 µg/ml (r^2>0.9996). The calculated limit of detection (LOD) and the limit of quantification (LOQ) were 0.025 and 0.05 µg/ml based on a signal-to-noise ratio of 3:1 and 6:1, respectively. The mean analytical recovery percentage of florfenicol in plasma was ranged from 97.5 to 99.7 %. The inter- and intra-day assay coefficients of variation ranged from 1.5 to 6.33 %. The accuracy values ranged from 98.4 to 102.8%.

Pharmacokinetic and statistical analysis

The pharmacokinetic analysis of the data was performed using non-compartmental methods based on statistical moment theory as previously described [26], using the commercially available software (Win Nonlin®, Pharsight Corporation, Cary, NC, US). The calculated parameters were: area under plasma concentration-time curve (AUC) and area under the first moment curve (AUMC), using the linear trapezoidal method; mean residence time (MRT), where MRT= AUMC/AUC; volume of distribution (Vd_z/F), where Vd_z = dose/AUC.β; elimination rate constant (k_{el}), which is the slope of the terminal log-linear portion of the plasma concentration-time profile, determined by least squares regression; AUC and AUMC extrapolated to infinity, by adding the ratio C_{last}/k_{el}; elimination half-life ($t_{1/2\beta}$), where $t_{1/2\beta}$ = 0.639/ k_{el}; total body clearance (Cl_B/F), where Cl_B = dose/AUC. The maximum concentration (C_{max}) and the corresponding peak time (t_{max}) were determined by inspection of the individual drug plasma concentration-time profiles.

Differences between the pharmacokinetic parameters of the two tested formulations were evaluated by one-way analysis of variance (ANOVA) using the commercially available software package (SPSS Inc., version 10.0, Chicago, IL, USA). Data are reported as mean ± SE. The differences were considered significant when P<0.05. AUC_{0-12h}, $AUC_{0-\infty}$ and C_{max} were considered to be the primary variables for

bioequivalence testing. The 90% CI for the ratio of test/reference (T/R) was calculated using WinNonlin® (Version 5.2, Pharsight Corporation, Cary, NC, USA).

Results

All tested chickens used in the present study were clinically healthy throughout the experimental period. Florfenicol (Flonicol® and Veterin®10%) was well tolerated by all chickens. Unexpected incidents that could have influenced the outcome of the study did not occur. The concentrations of florfenicol in chicken plasma were determined up to 12h and were not detected in all chickens 24h post single oral administration of both products. The mean concentration–time profile for florfenicol oral solutions is shown in Table 1 and Figure 1. The mean pharmacokinetic parameters of the two formulations after a single oral administration to broiler chickens at a dose of 20 mg/kg bw is shown in Table 2. The average means of AUC_{0-12}, $AUC_{0-\infty}$, C_{max} for Flonicol® and Veterin®10% were 26.45 ± 1.33 and 26.06 ± 1.20 µg.h/ml, 26.61 ± 1.33 and 26.26 ± 1.21 µg.h/ml, 9.02 ± 0.68 and 9.20 ± 0.77 µg/ml, respectively.

The results of the test for bioequivalence are shown in Table 3. The

Time post-administration (h)	Flonicol® (Test)	Veterin®10% (Reference)
0.166	2.24±0.50	2.11±0.57
0.33	4.42±0.96	5.89±1.12
0.5	6.80±1.16	7.95±1.24
0.75	7.02±1.10	7.92±1.16
1	7.38±0.90	7.43±0.86
1.5	7.02±0.65	6.42±0.44
2	5.99±0.55	5.38±0.37
4	2.54±0.21	2.51±0.38
6	1.02±0.17	0.99±0.19
8	0.53±0.18	0.34±0.08
10	0.13±0.03	0.14±0.02
12	0.08±0.01	0.10±0.02

Table 1: Mean plasma concentrations (µg/ml) of florfenicol (Flonicol® and Veterin®10%) in broiler chickens after a single oral dose of 20mg/kg bw. Values are mean ± SE (n=14).

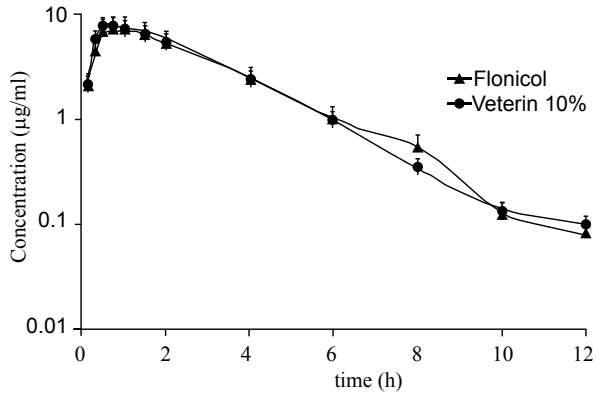

Figure 1: Semilogarthimic plot, showing the plasma concentrations–time profile of two florfenicol products (Flonicol® and Veterin®10%) after oral administration at a dose of 20 mg/kg bw as determined by HPLC method. Values are mean ± SE (n=14).

Parameters	Units	Formulations		P-value
		Flonicol® (Test)	Veterin®10% (Reference)	
C_{max}	µg/ml	9.02±0.68	9.20±0.77	0.839
t_{max}	h	1.02±0.13	1.05±0.30	0.899
$t_{1/2\beta}$	h	1.41±0.06	1.35±0.05	0.407
AUC_{0-12h}	µg.h/ml	26.45±1.33	26.06±1.20	0.803
$AUC_{0-\infty}$	µg.h/ml	26.61±1.33	26.26±1.21	0.821
AUMC	µg.h²/ml	71.78±4.65	69.98±8.80	0.832
MRT	h	2.72±0.18	02.62±0.27	0.723
Cl_B/F	ml\min\kg	12.82±0.63	12.96±0.60	0.844
Vd_z/F	l\kg	1.55±0.08	1.51±0.08	0.692

C_{max}, maximum plasma concentration; t_{max}, time to peak concentration; $t_{1/2\beta}$, elimination half-life; AUC_{0-12h}, area under plasma concentration-time curve from zero to 12h post drug administration; $AUC_{0-\infty}$, area under plasma concentration-time curve from zero to infinity; AUMC, area under the first moment time-concentration curve; MRT, mean residence time; F, systemic bioavailability; Cl_B/F, total body clearance/F; Vd_z/F, volume of distribution/F.

Table 2: Comparison of the mean plasma pharmacokinetic parameters obtained for florfenicol after single oral administration of Flonicol® (test) and Veterin®10% (reference) formulations to broiler chickens at 20mg/kg bw. Values are expressed as mean ± SE. (n=14).

Parameters	Units	90% Confidence interval	
		Lower bound (%)	Upper bound (%)
C_{max}	µg/ml	82.36	118.54
AUC_{0-12h}	µg.h/ml	91.86	111. 67
$AUC_{0-\infty}$	µg.h/ml	91.77	111.57

Two pharmaceutical formulations are bioequivalent when 90 % CI for the ratio of C_{max}, AUC_{0-12h} and $AUC_{0-\infty}$ between test and reference formulations fall between 80-125%.

Table 3: Bioequivalence between Flonicol® (test) and Veterin®10% (reference) formulations.

90% confidence interval of the ratios of the log-transformed values for AUC_{0-12h}, $AUC_{0-\infty}$, and C_{max} for the two formulations were within the acceptable bioequivalence range (80-125%).

Discussion

Florfenicol has been approved and become a valuable antibacterial in the treatment of serious bacterial infections in farm animals [16,27]. In poultry, florfenicol is used extensively for the twreatment of respiratory and gastrointestinal bacterial infections, administered via drinking water [7,17,24]. It has been reported that florfenicol showed greater activity than chloramphenicol and thiamphenicol, especially against Pasteurella, Salmonella, E. coli and Staphylococcus aureus [28]. Moreover, Florfenicol has superior pharmacological and pharmacokinetics features over some other antimicrobials used in chicken industry [18,29,30]. This drug is characterized by high bioavailability (F>80%), good tissue penetration and rapid elimination, which are important for the systemic treatment of domestic animals [7,15].

Several commercial local and international pharmaceutical preparations of florfenicol oral solution are currently available. In this respect, generic pharmaceutical preparations of florfenicol seeking approval to enter the market should demonstrate their ability to achieve C_{max} and AUC values that are equivalent to that of the original preparation. Inability to maintain high enough concentrations for sufficient periods of time may lead to therapeutic failure and may encourage the proliferation of resistant micro-organisms [19,23].

In this study, the pharmacokinetics and bioequivalence of two oral florfenicol solutions (Flonicol® and Veterin®10%) were investigated in healthy broiler chickens at a dose rate of 20mg/kg bw according to the manufacture's recommendations. In addition to the determination of the bioequivalence of both formulations, the results of the present experiment may contribute to the further understanding of the florfenicol plasma disposition kinetics in broiler chickens.

After a single oral administration of Flonicol® and Veterin®10% (20 mg/kg bw) to broiler chickens, both formulations were rapidly absorbed from the gastrointestinal tract and florfenicol was measurable at the first sampling time (10 min) in all chickens. The C_{max} was 9.02 ± 0.68 and 9.20 ± 0.77 μg/ml achieved at 01.02 ± 0.13 and 01.05 ± 0.30h (t_{max}) for Flonicol® and Veterin® 10%, respectively. The C_{max} obtained in the present study were similar to those reported previously in chickens at a dose level of 20mg/kg bw (10.23 ± 1.67 μg/ml) [31], higher than those reported in chickens at a dose level of 30 mg/kg bw (5.82 ± 2.43 and 3.20 ± 0.20 μg/ml) [15,24] similar to those reported in E. coli infected broiler chickens (7.9 ± 3.00 μg/ml) [17] and lower than those reported in turkeys (12.25 ± 2.62 μg/ml) [7]. The observed t_{max} was similar to those reported in healthy broiler chickens (1.05 ± 0.07h and 1.35 ± 0.43h) [15,16] and E. coli infected broiler chickens (1.1 ± 0.43h) [5] but shorter than those reported in turkeys (2.0 ± 1.22h) [7].

The lowest concentration of antimicrobials, which inhibits the growth of the target pathogen, is referred to the minimum inhibitory concentration (MIC) [32]. Florfenicol is considered as a bacteriostatic antibiotic whereby the efficacy is closely correlated with concentrations maintained above the MIC for a longer proportion of the interdosing interval (T > MIC) [33]. To our knowledge, the MICs of florfenicol for bacterial isolates from poultry have not yet been determined, and it is warranted for future study. In the current study, florfenicol plasma concentration was maintained above 0.1 μg/ml for 12h post drug administration for both products. Therefore, it is likely that florfenicol will need to be given twice a day at a dosage of 20 mg/kg bw to maintain therapeutic concentrations or continued in water 3-5 days.

The elimination half-life ($t_{1//2\beta}$) after oral administration of Flonicol® and Veterin®10% was 1.41 ± 0.06 and 1.35 ± 0.05h, respectively. The $t_{1//2\beta}$ was similar to those data reported in chickens (1.78 ± 0.19) [15] and E.coli infected broiler chickens (1.73 ± 0.25h) [17] and shorter than those reported in turkeys (3.76h) [7] and chickens in other studies (8.34 +/- 0.64h and 2.25 ± 0.53h) [13,31]. Differences between studies in chickens could be attributable to differences in the pharmacokinetic analysis (fitting to 1- versus 2-compartment models versus non-compartmental models).

This bioequivalence study was carried out in healthy broiler chicken under controlled conditions using a parallel design. The 90% confidence interval for the mean ratio of AUC_{0-12h}, $AUC_{0-\infty}$ and C_{max} (91.86-111.67, 91.77-111.57 and 82.36-118.54%, respectively) were within the European Agency for Evaluation of Medicinal Product (EMEA) and the US Food and Drug Administration (US-FDA) bioequivalence acceptance range of 80-125% (EMEA, 2006; FDA, 2006). Moreover, the average mean of C_{max}, t_{max}, AUC_{0-12h}, $AUC_{0-\infty}$, $t_{1/2\beta}$, Cl_B, MRT, Vd_z and AUMC were found to be very close with no significant difference between the two formulations. Based on the above pharmacokinetic and statistical results calculated in the current study, we concluded that Flonicol®, manufactured by Mobedco-Jordan, is bioequivalent to Veterin®10%, manufactured by Centrovet-Chile, and both products can be used as interchangeable drug in veterinary practice.

Aknowledments

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