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Pharmacokinetics and Bioequivalence of Two Norfloxacin Oral Dosage Forms (Vapcotril - 10%® and Mycomas 10%®) in Healthy Broiler Chickens

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Abstract: A pharmacokinetic and bioequivalence evaluation of two norfloxacin oral solutions was carried out in healthy broiler chickens after oral administration according to a single dose, randomized, parallel-experimental design. The two formulations were: Vapcotril-10%® (Vapco, Jordan) as a test product and Mycomas 10%® (Univet, Ireland) as a reference product. The chickens were allotted into 3 equal groups (8 chickens/group). Chickens of group 1 and 2 were given a single oral dose of Vapcotril-10%® and Mycomas 10%® at a dose level of 16 mg/kg body weight (bw), respectively after an overnight fasting. Chickens of group 3 were given a single intravenous dose of norfloxacin to calculate the systemic bioavailability. Serial blood samples were collected from the left brachial or cutaneous ulnar veins at different time points post drug administration. Norfloxacin concentrations in chicken plasma were determined using a microbiological assay and *Klebsiella pneumoniae* ATCC 10031 as a test organism. The pharmacokinetics analysis of the data was performed using non-compartmental analysis based on statistical moment theory (SMT) with the help of computerized WinNonlin program (Version 5.2, Pharsight, CA, USA). The maximum plasma concentration (C_{max}), time to reach peak concentration (t_{max}), area under plasma concentration-time curve (AUC_{0-12h} and AUC_{0-inf}), elimination half-life ($t_{1/2\beta}$) and systemic bioavailability (F) were. 4.94 ± 0.06 and 3.88 ± 0.07 μg/ml, 1.0 and 2.0 h, 21.60 ± 0.54 and 20.51 ± 0.39 μg.h/ml, 25.40 ± 0.76 and 23.40 ± 0.69 μg.h/ml, 4.49±0.13 and 3.87±0.21h, 50 and 47.5% for Vapcotril-10%® and Mycomas 10%®, respectively. The 90% confidence interval for test: reference ratio of the AUC_{0-12h} (99.53-111.15), AUC_{0-inf} (100.9-116.72) and C_{max} (122.69-132.15) was within the EMEA bioequivalence acceptance range of 80%–125% for the AUC and 75-133 for the C_{max}. In conclusion, Vapcotril-10%® is bioequivalent to Mycomas 10%® and can be used as interchangeable therapeutic agents in veterinary medicine practice.

Key words: Pharmacokinetics, bioequivalence, bioavailability, chicken and microbiological assay

Introduction

Norfloxacin is a flouroquinolone antibacterial agent that is extensively used in veterinary medicine practice (Prescott and Baggot, 1993; Sarkozy *et al*., 2004; Martinez *et al*., 2006; Cho *et al*., 2008). Norfloxacin has a wide range of antibacterial activity against most Gram-negative bacteria, some of Gram-positive aerobes as well as most *Mycoplasma* and *Chlamydia* Spp. (Ito *et al*., 1980; King *et al*., 1982; Norrby and Jonsson, 1983; Hannan *et al*., 1989). In addition, its antibacterial activity may extend to cover those organisms that are resistant to other antibacterial (Vancutsem *et al*., 1990; Neu and Labthavikul, 1982; Crumplin *et al*., 1984). Norfloxacin, like other flouroquinolone, exhibits a bactericidal effect which has been shown to be a concentration-dependent in its rate of killing with a post-antibiotic effect (PAE) against most Gram-negative pathogens (Neu *et al*., 1987). Norfloxacin is well absorbed after oral administration, highly lipid-soluble and penetrates tissues very rapidly and often reaching tissue concentrations higher than serum levels (Alestig, 1990). Because it's distinct pharmacological advantages and broad antibacterial spectrum, norfloxacin became a valuable antibiotic for the treatment of mycoplasmosis, colibacillosis and pasteurellosis in chickens (Laczay *et al*., 1998).

To obtain marketing approval, generic formulations must demonstrate bioequivalence to the marketed pioneer product in every target animal species included on the pioneer product label (Martinez *et al*., 2001). Bioequivalent drug products are generally statistically indistinguishable based on their respective concentration-time profiles for the active drug moiety in the appropriate biological matrix (Riviere, 1994).

Many studies that described the pharmacokinetics of norfloxacin in broiler chickens and turkeys are currently available (Anadon *et al*., 1992; Gulkaroc and Ziv, 1994; Ramadan *et al*., 1994; Laczay *et al*., 1998; Sarkozy *et al*., 2002 and 2004). However, limited published bioequivalence trails were reported. Accordingly, the aim of the current study is to evaluate the pharmacokinetic and bioequivalence of two norfloxacin oral dosage forms (Vapcotril® and Mycomas®) in healthy broiler chickens.

Materials and Methods

Study products

Test product: Vapcotril-10%®-Norfloxacin, 10% oral solution, Veterinary and Agricultural Products Manufacturing Co. Ltd. (VAPCO), Amman, Jordan.

Reference product: Mycomas 10%®-Norfloxacin, 10% oral solution, Univet, Ireland

The standard laboratory powder form of norfloxacin (99%) (Biochem-Pacific Import and Export Limited, Guangzhou, China) was dissolved in a sterile distilled water to give a final concentration of 50 mg/ml and used for intravenous administration only.

Experimental animals: Thirty two healthy broiler chickens (Hubbard x Hubbard) of 30-50 days old, weighing from 1.8-2.3 kg were used in this study. The chickens were purchased from local poultry farm. They were placed in the animal house at Jordan University of Science and Technology. The animals were monitored for 2 weeks for any apparent clinical signs 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.

Experimental design: The chickens were allotted into 3 equal groups (8 chickens/group). Chickens of group 1 and 2 were given a single oral dose of Vapcotril® and Mycomas® at a dose level of 16 mg/kg bw, respectively. Chickens of group 3 were given a single intravenous dose (16 mg/kg bw) of norfloxacin. Chickens were weighted prior drug administration and the doses were calculated accordingly. Norfloxacin was given directly into the crop using a thin plastic tube attached to a syringe for oral administration and in the right brachial vein for intravenous route. Food was withheld for 12 h before drug administration and was offered 6 h after drug administration. Water was provided *ad libitum*. The study was designed as randomized parallel protocol.

Sample collection: Blood samples (1-1.5 ml) were collected from the left brachial vein or cutaneous ulnar veins into heparinized tubes at 0 (pre-treatment), 5, 10, 15 and 30 min and at 2, 4, 2, 6, 8, 10, 12 and 24 h after intravenous administration and at 0, 10, 20, 30 min and 2, 4, 6, 8, 10, 12 and 24 hours after oral administration All blood samples were centrifuged directly at approximately 1000 g for 5 min and then the plasma was harvested and stored at -20 °C until assayed.

Assay procedure: Chicken plasma samples were assayed for determination of norfloxacin concentrations by microbiological assay as described previously (Bland *et al.*, 1983; Edberg, 1986) using *Klebsiella pneumoniae* ATCC 10031 as test organism and Mueller-hinton agar (Difco, Detroit, MI, USA). Five wells, 6mm in diameter, were made in a standard Petri-dish plate (120 mm) containing 25 ml inoculated agar. Wells were filled with test plasma samples or norfloxacin standard. Zones of inhibition were measured after 18 h of incubation at 37°C and the concentrations of norfloxacin were calculated from the standard curve. Standard curves of norfloxacin were prepared in antibacterial-free chicken plasma by the appropriate serial dilution. The standard curve in chicken plasma was linear over the range of 0.2 to 100 µg/ml (the correlation coefficient (R^2) = 0.997). The limit of quantification was 0.2 µg/ml.

Pharmacokinetic and statistical analysis: The pharmacokinetics analysis of the data was performed using non-compartmental analysis based on statistical moment theory (SMT) as previously described (Gibaldi and Perrier, 1982), with the help of computerized WinNonLin non-compartmental analysis program (version 5.2, Pharsight, USA). The calculated parameters were: Area under plasma concentration-time curve (AUC) using linear trapezoid method; mean residence time (MRT), where MRT= AUMC/AUC; volume of distribution based on terminal phase (Vd_z/F), where Vd_z = dose/AUC.β; elimination rate (K_{el}) was determined by least-square regression analysis of terminal log-linear portions of the plasma concentration-time profile (K_{el} = 2.303 x slop); 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 the inspection of the individual drug plasma concentration-time profiles. The absolute bioavailability (F) was calculated as (AUC_{non-IV} / AUC_{IV}) X 100.

For the purpose of bioequivalence analysis AUC_{0-12h}, AUC_{0-inf} and C_{max} considered as primarily variables after log-transformed using natural logarithms and submitted to ANOVA (WinNonlin program, Version 5.2, Pharsight, CA, USA) for parallel design and calculating standard 90% confidence intervals (CI) for the ratio of test/reference (T/R). All data are expressed as mean ± SE.

Results

The concentrations of norfloxacin in chicken plasma were determined up to 12 h and were not detected in all chickens 24 h post single oral and intravenous administration. The mean concentration-time profile for norfloxacin after oral and intravenous administration is shown in Table 1 and Fig. 1.

The mean pharmacokinetics parameters of norfloxacin (Vapcotril® and Mycomas®) after a single oral administration to broiler chickens at a dose of 16 mg/kg bw are shown in Table 2. The systemic bioavailability (F) was 50 and 47.5 % for Vapcotril® and Mycomas®, respectively. Following intravenous administration, the elimination half-life ($t_{1/2\beta}$), total body clearance (Cl_B),

Table 1: Mean plasma concentrations (µg/ml) of norfloxacin in broiler chickens after a single oral and intravenous administration (16 mg/kg bw). Values are mean ± SE (n=8)

Time post administration (h)	Vapcotril® (test)	Mycomas® (reference)	Intravenous
0.083	NA*	NA	28.47±1.04
0.166	0.65±0.02	0.57±0.02	18.54±0.92
0.25	NA	NA	16.44±0.82
0.33	1.16±0.02	1.00±0.05	NA
0.5	2.75±0.02	2.17±0.10	10.40±0.84
1	4.94±0.06	3.20±0.11	7.51±0.74
2	3.56±0.04	3.88±0.07	5.70±0.66
4	2.09±0.04	2.33±0.04	3.43±0.39
6	1.49±0.07	1.43±0.04	2.15±0.20
8	1.01±0.05	0.93±0.04	01.35±0.10
12	0.58±0.03	0.51±0.04	0.70±0.05
24	ND**	ND	ND

* NA: not applicable, **ND: not detected

Table 2: Mean plasma pharmacokinetic parameters obtained for norfloxacin in broiler chickens after a single oral and intravenous administration (16 mg/kg bw). Values are expressed as mean ± SE (n=8)

		Formulations		
Para-meters	Units	Vapcotril® (Test)	Mycomas® (Reference)	Intravenous
Cmax	µg/ml	4.94±0.06	3.88±0.07	-
t_{max}	h	1.00±0.00	2.00±0.00	-
$t_{1/2\beta}$	h	4.49±0.13	3.87±0.21	3.51±0.17
AUC_{0-12h}	µg.h/ml	21.60±0.54	20.51±0.39	43.17±3.68
AUC_{0-inf}	µg.h/ml	25.40±0.76	23.40±0.69	46.64±3.77
MRT	h	4.05±0.05	4.12±0.06	2.96±0.04
Cl_B/F	ml\min\kg	10.57±0.31	11.46±0.33	6.00±0.51
Vd_z/F	l\kg	4.11±0.16	3.81±0.12	01.87±0.24
V_{ss}	l/kg	-	-	1.46±0.14
F	%	50	47.5	-

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 12 h post drug administration; AUC_{0-inf}, area under plasma concentration-time curve from zero to infinity; MRT, mean residence time; Cl_B/F, total body clearance/F; Vd_z/F, volume of distribution based on terminal phase; V_{ss}, volume of distribution at steady-state; F, mean systemic bioavailability.

Table 3: Bioequivalence between Vapcotril® (test) and Mycomas® (reference) formulations

		90% Confidence interval		
Para-meters	Units	Lower bound(%)	Upper bound(%)	Power
C_{max}	µg/ml	122.69	132.15	1
AUC_{0-12h}	µg.h/ml	99.53	111. 15	0.981
AUC_{0-inf}	µg.h/ml	100.9	116.72	0.981

Two pharmaceutical formulations are bioequivalent when the ratio between test and reference formulations results between 80-125% for both AUC_{0-12h} and AUC_{0-inf} and between 75-133% for C_{max} (EMEA, 2006).

apparent volume of distribution (Vd_z) and the volume of distribution at steady-state (V_{SS}) was 3.51±0.17, 6.00±0.51, 6.00±0.51 and 1.87±0.24, respectively (Table 2).

The average means of AUC_{0-12}, AUC_{0-inf} and the C_{max} were 21.60±0.54 and 20.51±0.39 µg.h/ml, 25.40±0.76 and 23.40±0.69 µg.h/ml, 4.94±0.06 and 3.88±0.07 µg/ml for Vapcotril® and Mycomas®, respectively. The mean and standard error (SE) of AUC_{0-12h}, AUC_{0-inf} and C_{max} for the two products were found very close, indicating that the plasma profiles generated by Vapcotril® are comparable to those produced by Mycomas®. The 90% confidence intervals demonstrated that the ratios of AUC_{0-12h}, AUC_{0-inf}, and C_{max} of the two formulations were 99.53-111.15, 100.9-116.72, 122.69-132.15, respectively (Table 3).

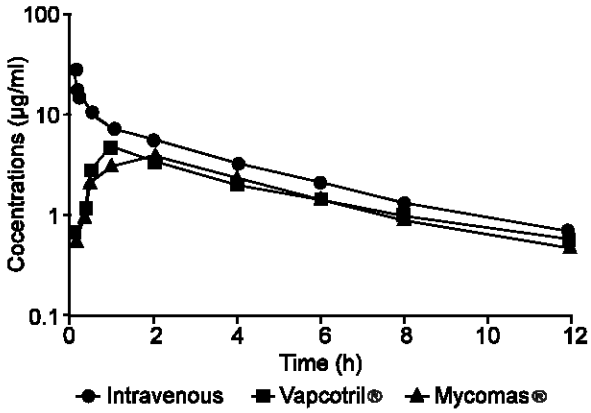

Fig. 1: Semilogarthimic plot, showing the plasma concentrations - time profile of norfloxacin after oral and intravenous administration at a dose of 16 mg/kg bw as determined by microbiological assay. Values are mean ± SE (n=8).

Discussion

Norfloxacin, is one of the first modern flouroquinolone antimicrobial agents featuring a fluorine atom at C-6 and a piperazine ring at C-7, has increased its potency in relation to other flouroquinolone (Wentland, 1990; Samanidou *et al.*, 2003). In the current study, we investigated the pharmacokinetics and bioequivalence of two oral norfloxacin solutions (Vapcotril-10%® and Mycomas10%®) in healthy broiler chickens at a dose rate of 16 mg/kg bw. Norfloxacin concentrations in chicken plasma samples were determined by microbiological assay method that has been previously published (Bland *et al.*, 1986). This microbiological method does not differentiate between the antimicrobial activity associated with a parent compound or its metabolites (e.g. desethylenenorfloxacin and oxonorfloxacin) or even other microbial growth inhibitors that could be present in a given formulation. Nevertheless, it measures the total activity which could be more useful for pharmacodynamic evaluations than high performance liquid chromatography (HPLC) methods (McKellar *et al.*, 1999; Heinen, 2002).

After a single oral administration of Vapcotril® and Mycomas® at a dose of 16 mg/kg bw to broiler chickens, both formulations were rapidly absorbed from the

gastrointestinal tract (GIT) and norfloxacin was measurable at the first sampling time (10 min) in all tested chickens with absolute bioavailability of 50 and 47.5%, respectively. The maximum plasma concentrations (C_{max}) were 4.94±0.06 and 3.88±0.07 µg/ml being achieved at 1.0 and 2.0 h (t_{max}) for Vapcotril® and Mycomas®, respectively. The C_{max} obtained in the present study were higher than those reported in healthy broiler chickens (1.46 ± 0.18 µg/ml), turkeys (0.95 ± 0.15 µg/ml) and geese (1.58 ± 0.3 µg/ml) given norfloxacin at a dose level of 10 mg/kg bw (Laczay *et al*., 1998). The difference in the dose level in these studies may contribute to the differences in the C_{max}. However, our achieved t_{max} for both formulations was in agreement with those reported in broiler chickens, turkeys and geese (1-2 h) (Laczay *et al*., 1998). The lowest concentration of antimicrobials, which inhibit the growth of the target pathogen, is referred to the minimum inhibitory concentration (MIC) (Salmon and Watts, 2000). The MIC_{90} of norfloxacin against the major poultry pathogenic Gram-negative bacteria (*E. coli*, *Salmonella* spp., *Pasteurella* spp.) and mycoplasmas is about 0.25 µg/ml (Hannan *et al*., 1989; Prescott and Baggot, 1993; Laczay *et al*., 1998). In the present experiment, Vapcotril® and Mycomas® plasma concentrations exceeding 0.25 µg/ml were maintained for up to 12 h after a single oral administration of both products. Therefore, norfloxacin should be given twice a day at a dose level of 8 mg/kg bw to maintain therapeutic concentrations or continues in water 3-5 days.

Following single intravenous administration, the elimination half-life ($t_{1/2\beta}$) (3.51±0.17 h) of norfloxacin was shorter than those previously reported in chicken (8.0±0.3) (Anadon *et al*., 1992). The total body clearance (Cl_B) was 6.0±0.51 ml/min/kg. Whereas, the apparent volume of distribution (Vd_z) and the volume of distribution at steady state (V_{ss}) calculated in the current experiment were 1.87±0.24 and 1.46±0.14 l/kg, respectively, indicting a wide norfloxacin distribution in tissues.

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 AUC_{0-12h}, AUC_{0-inf} and C_{max} were 99.53-111.15%, 100.9-116.72% and 122.69-132.15%, respectively. These values falls within the EMEA bioequivalence acceptance range of 80-125% for the AUC and 75-133% for the C_{max} (EMEA, 2006). Based on the above pharmacokinetic and statistical results calculated in the current study, we concluded that Vapcotril®, manufactured by Vapco-Jordan, is bioequivalent to Mycomas®, manufactured by Univet-Ireland and both products can be used as interchangeable drug in veterinary medicine practice.

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