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 (Cmax), time to reach peak concentration (tmax), area under plasma concentration-time curve (AUC0-12h and AUC0-∞), elimination half-life (t1/2) 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-∞ (100.9-116.72) and Cmax (122.69-132.15) was within the EMEA bioequivalence acceptance range of 80%-125% for the AUC and 75-133 for the Cmax. 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 fluoroquinolone 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; Norby 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 fluoroquinolone, 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, 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 (R²) = 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/F), where Vd = dose/AUC; elimination rate (K) was determined by least-square regression analysis of terminal log-linear portions of the plasma concentration-time profile (K= 2.303 x slop); elimination half-life (t1/2), where t1/2 = 0.639/ K; total body clearance (Cl/F), where Cl = dose/AUC; The maximum concentration (Cmax) and the corresponding peak time (tmax) were determined by the inspection of the individual drug plasma concentration-time profiles. The absolute bioavailability (F) was calculated as (AUCoral / AUCiv) X 100.

For the purpose of bioequivalence analysis AUC0-12h, AUC0-24h and Cmax 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 (t1/2), total body clearance (Cl),...
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)

<table>
<thead>
<tr>
<th>Time post administration (h)</th>
<th>Vapcotril® (Test)</th>
<th>Mycomas® (Reference)</th>
<th>Intravenous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cmax µg/ml</td>
<td>tmax h</td>
<td>MRT h</td>
</tr>
<tr>
<td>0.083</td>
<td>4.94±0.06</td>
<td>1.00±0.00</td>
<td>4.05±0.05</td>
</tr>
<tr>
<td>0.166</td>
<td>6.5±0.02</td>
<td>2.17±0.10</td>
<td>7.5±0.74</td>
</tr>
<tr>
<td>0.25</td>
<td>NA</td>
<td>3.20±0.11</td>
<td>3.87±0.21</td>
</tr>
<tr>
<td>0.33</td>
<td>1.16±0.02</td>
<td>3.88±0.07</td>
<td>5.7±0.66</td>
</tr>
<tr>
<td>0.5</td>
<td>2.75±0.02</td>
<td>2.33±0.04</td>
<td>3.43±0.39</td>
</tr>
<tr>
<td>1</td>
<td>4.94±0.06</td>
<td>3.88±0.07</td>
<td>4.11±0.16</td>
</tr>
<tr>
<td>2</td>
<td>3.56±0.04</td>
<td>3.88±0.07</td>
<td>3.81±0.12</td>
</tr>
<tr>
<td>4</td>
<td>2.09±0.04</td>
<td>3.88±0.07</td>
<td>0.51±0.04</td>
</tr>
<tr>
<td>6</td>
<td>1.49±0.07</td>
<td>3.88±0.07</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>1.01±0.05</td>
<td>3.88±0.07</td>
<td>0.70±0.05</td>
</tr>
<tr>
<td>12</td>
<td>0.58±0.03</td>
<td>3.88±0.07</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>ND</td>
<td>3.88±0.07</td>
<td>-</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vapcotril® (Test)</th>
<th>Mycomas® (Reference)</th>
<th>Intravenous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax µg/ml</td>
<td>4.94±0.06</td>
<td>3.88±0.07</td>
<td>-</td>
</tr>
<tr>
<td>tmax h</td>
<td>1.00±0.00</td>
<td>2.00±0.00</td>
<td>-</td>
</tr>
<tr>
<td>t1/2&lt;sub&gt;ss&lt;/sub&gt; h</td>
<td>4.49±0.13</td>
<td>3.87±0.21</td>
<td>3.51±0.17</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-12h&lt;/sub&gt; µg/h/ml</td>
<td>21.60±0.54</td>
<td>20.51±0.39</td>
<td>30.26±1.14</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-inf&lt;/sub&gt; µg/h/ml</td>
<td>25.40±0.76</td>
<td>23.40±0.69</td>
<td>46.64±3.77</td>
</tr>
<tr>
<td>MRT h</td>
<td>4.05±0.05</td>
<td>4.12±0.06</td>
<td>2.96±0.04</td>
</tr>
<tr>
<td>Cl/F ml/min/kg</td>
<td>10.57±0.31</td>
<td>11.46±0.33</td>
<td>6.00±0.51</td>
</tr>
<tr>
<td>V&lt;sub&gt;d&lt;/sub&gt;/F l/kg</td>
<td>4.11±0.16</td>
<td>3.81±0.12</td>
<td>0.18±0.24</td>
</tr>
<tr>
<td>V&lt;sub&gt;s&lt;/sub&gt; l/kg</td>
<td>-</td>
<td>-</td>
<td>1.46±0.14</td>
</tr>
<tr>
<td>F %</td>
<td>50</td>
<td>NA</td>
<td>47.5</td>
</tr>
</tbody>
</table>

Discussion

Norfloxacin, is one of the first modern fluoroquinolone antimicrobial agents featuring a fluorine atom at C-6 and a pipera zine ring at C-7, has increased its potency in relation to other fluoroquinolone (Wentland, 1990; Samanidou et al., 2003). In the current study, we investigated the pharmacokinetics and bioequivalence of two oral norfloxacin solutions (Vapcotril-10% and Mycomas®) in healthy broiler chickens at a dose rate of 16 mg/kg bw. Norfloxacin concentrations in chicken plasma samples were determined by微生物ological 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
Bioequivalence of Two Oral Norfloxacin Formulations

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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 (Cmax) were 4.94±0.06 and 3.88±0.07 µg/ml being achieved at 1.0 and 2.0 h (tmax) for Vapcotril® and Mycomas®, respectively. The Cmax 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 Cmax. However, our achieved tmax 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 MIC90 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 (t1/2) (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 (Clb) was 6.0±0.51 ml/min/kg. Whereas, the apparent volume of distribution (Vd) and the volume of distribution at steady state (Vss) calculated in the current experiment were 1.87±0.24 and 1.46±0.14 l/kg, respectively, indicating 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 AUC0-12h/AUC0-inf and Cmax 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 Cmax (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.

Acknowledgment

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References


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