Comparative serum concentrations and pharmacokinetics of levofloxacin and danofloxacin in broiler chickens

K. Abo-EL-Sooud*; Ahmed M. Soliman; A. Goudah and Sarah Fayez Sobhy

Pharmacology Department, Faculty of veterinary Medicine, Cairo University, Giza/Egypt. P.O. Box 12211, Giza, Egypt. Fax: 0020235725240 – 002035710305

K. Abo-EL-Sooud (Corresponding author)
Pharmacology Department, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.
Tel: +201066756870 E-mail: kasooud@cu.edu.eg

Abstract

The serum concentrations and pharmacokinetics of levofloxacin and danofloxacin in broiler chickens was compared following single intravenous (IV) or oral administration at 10 mg/kg of body weight (b.w.). Serum concentrations of levofloxacin and danofloxacin were determined by specific and sensitive high performance liquid chromatography (HPLC) methods. Pharmacokinetic parameter values for both fluoroquinolones were calculated by non-compartmental analysis. After IV injection, the elimination half-life ($T_{1/2\beta}$) was twofold higher and the mean residence time (MRT) was threefold higher for danofloxacin compared to levofloxacin. The values for total body clearance ($Cl_B$) were 13.67 vs. 33.86 ml/min/kg and volume of distribution at steady state ($V_{dss}$) were 6.84 vs. 59.78 L/kg. However, area under the serum concentration vs. time curve of levofloxacin was greater than for danofloxacin. Maximum plasma concentration ($C_{max}$) after oral administration was 1.41 and 0.59 $\mu$g/ml for levofloxacin and danofloxacin, attaining at one and two hours for both drugs, respectively. Systemic bioavailability ($F$) was 89% for levofloxacin and $< 100\%$ for danofloxacin. Furthermore, the apparent volume of distribution ($V/F$) of danofloxacin was (37.92 L/kg) significantly higher than that of levofloxacin (15.40 L/kg). Following oral administration, the $C_{max}$/MIC ratio of 14.11 and 6.85 and AUC/MIC ratio of 108.68 and 52.36, for levofloxacin and danofloxacin, respectively, indicates potential clinical and bacteriological efficacy of levofloxacin. Based on these parameters, a dose of 10 mg/kg b.w. of levofloxacin given orally every 24 h in chickens can maintain effective serum concentrations with bacterial infections with MIC$_{90}$ > 0.1 $\mu$g/ml.

Keywords: Pharmacokinetics, Levofloxacin, Danofloxacin, Chickens, HPLC.
1. Introduction

In veterinary clinical practice the sensitivity of a given animal species to a certain drug can be attributed to pharmacodynamic and pharmacokinetic variations. In contrast to human medicine where individual differences are of primary importance, interspecies and also inter-breed distinctions are crucial in comparative veterinary medicine (Jerzsele, 2012). Increasing worry has been expressed about the extensive use of fluoroquinolones in poultry, as resistant zoonotic organisms may transmit to human. A relationship has been reported between septicaemic human and animal pathogenic bacterial strains (Johnson et al., 2002), confirming the demand for appropriate use of these compounds. The ability to attain clinical efficacy and to minimize the spread of resistant pathogens is a correlation between the pharmacokinetic and pharmacodynamic behaviors of fluoroquinolones (Wise, 2003). Levofloxacin and danofloxacin belong to the group of synthetic fluoroquinolone compounds developed mainly for veterinary use (Garcia et al., 2000). They act predominantly by inhibiting the enzyme topoisomerase II, hence suppressing DNA and RNA replication. Fluoroquinolones result in concentration-dependent killing of many Gram-negative microorganisms (Aliabadi and Lees, 2001; Sarasola et al., 2002) as well as atypical pathogens such as Mycoplasma and Chlamydia (Eliopoulos et al., 1996). Fluoroquinolones show rapid and extensive tissue distribution in interstitial fluid, skin and bones are 35-100% of those obtained in serum, where as bronchial secretions and prostatic concentrations are two to three times of corresponding serum concentrations (Jerzsele, 2012). Penetration into CSF is approximately 25% of serum concentration (Davis et al., 2006). Fluoroquinones attain high intracellular concentrations in macrophages and neutrophils. Intracellular concentrations are 4-10 times greater than plasma concentrations. The pharmacokinetics of levofloxacin and danofloxacin has been investigated in avian species including pheasants, guinea fowls and Japanese quails (Dimitrova et al., 2014), turkeys (Haritova et al., 2006), ducks (Aboubakr and Soliman, 2014), chickens (Knoll et al., 1999; El-Gendi et al., 2001, Deng et al., 2003). However, there is little information concerning comparative clinical study to recommend the most efficacious fluoroquinones in poultry industry. Consequently, the aim of this investigation was to compare serum concentrations and pharmacokinetics of levofloxacin and danofloxacin inbroiler chickens following single intravenous or oral administration at 10 mg/kg b.w.
2. Materials and methods

2.1. Chickens

Female broiler chickens (Hubbard breed), 40–45 days old, weighing between 2 and 2.5 kg, were obtained. During acclimatization (at least 2 weeks before starting the experiment to ensure the complete withdrawal of any residual drugs) and subsequent treatment periods, all chickens had free access to water and antibacterial-free food. The animal house temperature was maintained at 22±2°C and humidity at 40–55%. The study was approved by the Animal Care and Use Committee at the Faculty of Veterinary Medicine, Cairo University. The animals were allowed to acclimatize and did not receive any drug treatment for at least 15 days preceding the study.

2.2. Experimental design

Twenty broiler chickens were divided into four groups of five each. Chickens in group-I and II were administered levofloxacin (10 mg/kg body weight) intravenously and orally, while chickens in group-III and IV received similar dose of danofloxacin (10 mg/kg body weight). The intravenous injection was done in left wing vein and oral administration by using thin plastic tube attached to a syringe into the crop. Blood samples (0.5 ml each) were taken via indwelling catheter into Vacutainers (Becton Dickinson vacutainer Systems, Rutherford, NJ, USA), from the right wing vein at 0 (blank sample), 0.166, 0.25, 0.33, 0.5, 0.75, 1, 2, 4, 6, 8, 10, 12, 18, 24, 48 and 72 h in all groups. Serum was separated by centrifugation at 3000g for 10 min and stored at -20°C until assayed.

2.3. Analysis for levofloxacin and danofloxacin

The serum concentrations of levofloxacin and danofloxacin were determined by a reverse phase high-performance liquid chromatography (HPLC). 50 µL supernatant was injected into the HPLC system for analysis. Levofloxacin serum concentrations’ were determined according to the method of Ishiwata et al., 2007. Chromatography was carried out using a Hypersil C18 column (5 µm, 4.6 mm x 250 mm); the mobile phase consisted of 12% acetonitrile, 0.75% formic acid, and 0.4% triethylamine, while the UV detector was
operated at a wavelength of 295 nm. The analytical method was validated by limit of quantification, linearity for a range of concentrations, accuracy, precision, and susceptibility to interferences.

Danofloxacin serum concentrations’ were determined according to the method of Hubicka et al., 2014. Chromatography was performed on a Gemini-NX C18 110A, 150 mm 4.60 mm, 3 mm particle size column with 0.025 M phosphate buffer (pH= 5)–acetonitrile–methanol (95 : 10 : 30 v/v/v) as the mobile phase at a flow rate of 1.2 ml/min. UV detection was performed at 280 nm. The column was adjusted at 25 °C.

2.4. Sample preparation

To a 200 µl aliquot of chicken serum, 100 µl of 20% perchloric acid was added. The mixture was vortexed, and then centrifuged at 4500 rpm for 10 min. A 100 µl aliquot of the supernatant solution was added to the auto-sampler vial for analysis.

2.5. Methods validation

The calibration curves of serum were prepared with seven different concentrations between 0.001 and 10 µg/ml using blank chicken serum. A calibration curve was obtained by plotting the peak area versus the nominal concentrations. The equation was calculated by the least-squares method using linear regression. The standard curves of levofloxacin and danofloxacin in chicken serum were linear, lying between 0.001 and 5 µg/ml ($r^2 > 0.98$). The peak area of unknown specimen was compared with that of the standards levofloxacin and danofloxacin. The precision and accuracy of the method were evaluated by repetitive analysis of the serum samples (n=6) spiked with different known concentrations of both tested drugs. Intra-assay variations were determined by measuring seven replicates of three standard samples used for calibration curves. The intra-assay variation coefficients were < 6% for serum. An inter-assay precision was determined by assaying the three standard samples on three separate days. The inter-assay variation coefficients were <5% for serum. Recovery from serum was found to be 98%. The limit of quantification (LOQ) for serum was 0.001 µg/ml for levofloxacin and danofloxacin.
2.6. Pharmacokinetic analysis

Serum concentrations of levofloxacin and danofloxacin after IV and oral administrations were subjected to non-compartmental analysis using a linear pharmacokinetic model with the help of a PK Solver, China Pharmaceutical University, Nanjing, Jiangsu, China (Zhang et al., 2010) a freely available menu-driven add-in program for Microsoft Excel written in Visual Basic for Applications (VBA), for solving basic problems in pharmacokinetic (PK) and pharmacodynamic (PD) data analysis. Maximum serum concentration (C_{max}) in serum and the time required to reach it (T_{max}) were directly calculated from the data by the software program. The elimination rate constant was estimated by log-linear regression of concentrations observed during the linear phase of elimination, using at least three data points automatically selected by the program and confirmed by visual examination of the plotted data. The area under the concentration vs. time curve (AUC_{0-\infty}) was calculated using the linear trapezoidal rule and absolute bioavailabilities (F) were calculated according to the following equations:

\[ F = \frac{\text{mean AUC}_{\text{oral}}}{\text{mean AUC}_{\text{IV}}} \times 100. \]

2.7. Statistical analysis

The statistical analysis was performed using the SPSS® 10.0 software package (SAS, Cary, NC, USA). Results are presented as arithmetic mean ± standard error (S.E.). The nonparametric Wilcoxon test was used to compare the parameters obtained for levofloxacin and danofloxacin following both routes of administration. Means were considered significantly different at p< 0.05 and P<0.01.

3. Results

After IV injections serum concentrations of levofloxacin and danofloxacin are presented in Figure 1 and a summary of the kinetic parameters are given in Table I. The elimination half-life (T_{1/2}) was twofold higher and the mean residence time (MRT) was threefold higher for danofloxacin compared to levofloxacin. However, values for total body clearance (Cl_{B}) were 13.67 vs. 33.86 ml/min.kg and volume of distribution at steady state (V_{dss}) were 6.84 vs. 59.78 L/kg, respectively.
The mean ± S.E. serum levofloxacin and danofloxacin concentrations after oral administration at a dose of 10 mg/kg are presented in Figure 2. Data for serum were also analyzed by non-compartmental analysis and the obtained kinetic parameters are shown in Table 2. Maximum plasma concentration (C\text{max}) after oral administration was 1.41 and 0.59 µg/ml for levofloxacin and danofloxacin, attaining at 1 and 2 h for both drugs, respectively. Furthermore, the apparent volume of distribution (V/F) of danofloxacin was (37.92 L/kg) significantly higher than that of levofloxacin (15.40 L/kg). Both drugs were detected up to 48 h post oral administrations. Bioavailability (F) was high: 89% for levofloxacin and < 100% for danofloxacin indicating excellent absorption from gastrointestinal tract.

4. Discussion

Fluoroquinolones are class of carboxylic acid derivatives, exhibits bactericidal activity against numerous Gram-negative and some Gram-positive bacteria, mycoplasmas and intracellular pathogens, such as Brucella and Chlamydia species (Hannan et al., 1997; Sarasola et al., 2002). They have been registered for use in veterinary medicine, including for treatment of bacterial infection in avian species (Fiorentin et al., 2003). There are substantial differences among these drugs with regards to the extent and rate of biotransformation, routes of excretion, and elimination half lives. Improper use of these modern agents can potentially lead to bacterial resistance and thereby remove them from the veterinarian’s focus of chemotherapeutic agents. The elimination half life and mean residence time of danofloxacin were significantly longer as compared to levofloxacin. In this respect, Varia et al., 2009 stated that levofloxacin is more rapidly eliminated than other fluoroquinolones in broiler chickens. Moreover, Dimitrova et al., 2014 stated that danofloxacin is eliminated mainly unchanged and relatively slowly, providing a 24 h therapeutic concentration.

The volumes of distribution at steady state (V\text{dss}) were 6.84 vs. 59.78 L/kg, respectively. Danofloxacin, like other fluoroquinolones, is adequately lipid-soluble to penetrate tissues which are supported by high value of volumes of distribution. Higher concentrations of danofloxacin in tissues than in serum of broiler chickens (Anadon et al., 1997; Knoll et al., 1999; El-Gendi et al., 2001), turkeys (Haritova et al., 2006) and ducks (Goudah and Mounier, 2009). Yang et al., 2014 assumed that danofloxacin was mainly eliminated from renal excretion and hepatic metabolism and both Cl\text{e} and Cl\text{he} were used to simulate these elimination processes. Both values were 0.31 ± 0.03 and 0.11 ± 0.02 L/h/kg, respectively, which indicated that the total
body clearance (Cl\textsubscript{tot}) of danofloxacin was 0.42 L/h/kg. In the present study the total body clearance (Cl\textsubscript{tot}) of danofloxacin was 2.03 L/h/kg was faster to those reported in infected chickens (Yang \textit{et al.}, 2014) and turkeys (0.59 ± 0.14 L/h/kg; Haritova \textit{et al.}, 2006), but close to those in Japanese quail (1.61 ± 0.14 L/h/kg; Dimitrova \textit{et al.}, 2014; 1.48 ± 0.17 L/h/kg; Haritova \textit{et al.}, 2013), and Guinea fowl (1.23 ± 0.07 L/h/kg; Dimitrova \textit{et al.}, 2014). This might be because of the increases of blood flow through liver and kidney during infection. Following oral administration the peak serum concentration of danofloxacin was significantly greater than the concentrations of danofloxacin (1.41 and 0.59 µg/ml). However, area under the serum concentration vs. time curve of levofloxacin was greater than for danofloxacin.

The systemic bioavailability of levofloxacin (89 %) was higher than bioavailability of levofloxacin in chickens (59.54%, Varia \textit{et al.}, 2009) and ducks (73.65%, Aboubakr and Soliman, 2014). Systemic bioavailability (F) was <100% for danofloxacin, this value is in good agreement with the values of 102% reported by Lynch \textit{et al.}, 1994 and 99.2% reported by Knoll \textit{et al.}, 1999 in chickens. It was assumed that all administrated danofloxacin was immediately available in gastrointestinal tract. The absorbed drug was assumed to go directly into the liver via the portal vein, and the unabsorbed drug was excreted in feces with the intestinal elimination rate constant.

The use of antibacterials in poultry is often associated with incomplete bacterial eradication, resulting in an insufficient clinical response in some cases and the risk of the emerge of antibacterial resistance (Haritova \textit{et al.}, 2006). Knowledge of the PK and PD properties of danofloxacin, obtained by a PK-PD approach, can be applied to evaluate dosing regimens. Based on many \textit{in vitro} and \textit{in vivo} studies performed previously, it has been established that fluoroquinolones are concentration dependant antibacterial agents, the AUC/MIC ratio is the most important factor in predicting efficacy, with the rate of clinical success being greater than 80%, when this ratio is higher than 100–125 (Lode \textit{et al.}, 1998). A second predictor of efficacy for concentration dependent antibiotic is the ratio C\textsubscript{max}/MIC, considering that values above 8–10 would lead to better clinical results and to avoidance of bacterial resistance emergence (Walker, 2000). Levofloxacin pharmacokinetic/pharmacodynamic integration revealed significantly higher values for C\textsubscript{max}/MIC and AUC/MIC ratios in healthy ducks, indicating the excellent pharmacokinetic characteristics of the drug. To cover most of the susceptible organisms, in this discussion, the MIC\textsubscript{90} of 0.032–0.5 µg/mL was reported as minimum therapeutic concentration (MIC\textsubscript{90}) for levofloxacin against most bacteria (Chulavatnatol \textit{et al.}, 2001).
An average MIC$_{90}$ of 0.1 µg/mL of levofloxacin has been taken into consideration for calculation of efficacy predictors. Following oral administration, the C$_{max}$/MIC ratio of 14.11 and 6.85 and AUC/MIC ratio of 108.68 and 52.36, for levofloxacin and danofloxacin, respectively, indicates potential clinical and bacteriological efficacy of levofloxacin. Based on these parameters, a dose of 10 mg/kg b.w. of levofloxacin given orally every 24 h in chickens can maintain effective serum concentrations with bacterial infections with MIC$_{90} > 0.1$ µg/ml.

5. References


<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Levofloxacin</th>
<th>Danofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$</td>
<td>h$^{-1}$</td>
<td>0.074 ± 0.03</td>
<td>0.037 ± 0.01</td>
</tr>
<tr>
<td>t$_{1/2\beta}$</td>
<td>h</td>
<td>9.39 ± 1.12</td>
<td>18.49 ± 2.45**</td>
</tr>
<tr>
<td>C$_{p0}$</td>
<td>µg/ml</td>
<td>4.35 ± 0.95</td>
<td>0.87 ± 0.21*</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$</td>
<td>µg•h/ml</td>
<td>12.19 ± 2.03</td>
<td>4.92 ± 0.75*</td>
</tr>
<tr>
<td>AUMC</td>
<td>µg•h$^2$/ml</td>
<td>101.66 ± 7.45</td>
<td>144.81 ± 11.12</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>8.34 ± 1.39</td>
<td>29.42 ± 3.65**</td>
</tr>
<tr>
<td>V$_C$</td>
<td>L/kg</td>
<td>2.30 ± 0.55</td>
<td>11.74 ± 1.76**</td>
</tr>
<tr>
<td>Cl$_{tot}$</td>
<td>ml/min/kg</td>
<td>13.67 ± 2.11</td>
<td>33.86 ± 3.98**</td>
</tr>
<tr>
<td>V$_{ds}$</td>
<td>L/kg</td>
<td>6.84 ± 1.34</td>
<td>59.78 ± 7.14**</td>
</tr>
</tbody>
</table>

$\beta$: Elimination rate constant; T$_{1/2\beta}$: elimination half-life; C$_{p0}$ = The concentration of drug in serum at time zero time; V$_C$: Volume of distribution of central compartment; V$_{ds}$: volume of distribution at steady state; Cl$_{tot}$: total body clearance; AUC: area under the curve by the trapezoidal integral; AUMC: area under moment curve by the trapezoidal integral; MRT. Values of curcumin treated chickens were significantly different from corresponding normal chickens at *$P$<0.05, **$P$<0.01.
Table (2): Mean pharmacokinetic parameters for levofloxacin and danofloxacin (10 mg/kg b.w.) after oral administration to broiler chickens (n=5)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Levofloxacin</th>
<th>Danofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{el}$</td>
<td>h$^{-1}$</td>
<td>0.06 ± 0.01</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>$t_{1/2el}$</td>
<td>h</td>
<td>11.60 ± 1.73</td>
<td>13.76 ± 1.66</td>
</tr>
<tr>
<td>$T_{max}$</td>
<td>h</td>
<td>1.00 ± 0.15</td>
<td>2.00 ± 0.43</td>
</tr>
<tr>
<td>$C_{max}$</td>
<td>μg/ml</td>
<td>1.41 ± 0.22</td>
<td>0.58 ± 0.13*</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$</td>
<td>μg•h/ml</td>
<td>10.87 ± 2.25</td>
<td>5.24 ± 1.44*</td>
</tr>
<tr>
<td>AUMC</td>
<td>μg•h$^2$/ml</td>
<td>152.33 ± 17.84</td>
<td>81.66 ± 6.23*</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>14.02 ± 2.13</td>
<td>15.60 ± 3.45</td>
</tr>
<tr>
<td>V/F</td>
<td>L/kg</td>
<td>15.40 ± 2.97</td>
<td>37.92 ± 4.88*</td>
</tr>
<tr>
<td>Cl/F</td>
<td>L/h/kg</td>
<td>0.92 ± 0.14</td>
<td>1.91 ± 0.31*</td>
</tr>
<tr>
<td>F</td>
<td>%</td>
<td>89.16 ± 3.23</td>
<td>&lt; 100</td>
</tr>
</tbody>
</table>

$k_{el}$: Elimination rate constant; $T_{1/2el}$: elimination half-life; $T_{max}$: time to peak concentration; $C_{max}$: maximum serum concentration; AUC: area under the curve by the trapezoidal integral; AUMC: area under moment curve by the trapezoidal integral; MRT: mean residence time; Cl/F = apparent clearance; V/F = apparent volume of distribution; F%: bioavailability. Values of curcumin treated chickens were significantly different from corresponding normal chickens at *$P<0.05$. 
Figure 1. Mean ± S.E. serum concentrations of levofloxacin and danofloxacin (10 mg/kg body weight) in broiler chickens after single intravenous bolus administration.
Figure 2. Mean ± S.E. serum concentrations of levofloxacin and danofloxacin (10 mg/kg body weight) in broiler chickens after single oral bolus administration.