Pharmacokinetics of difloxacin in Japanese quails (Coturnix japonica) after single intravenous and oral administration

M. Aboubakr, M. Elbadawy⁎

Department of Pharmacology, Faculty of Veterinary Medicine, Benha University, 13736, Moshtohor, Toukh, Elqaliobiya, Egypt

ARTICLE INFO

Keywords:
Difloxacin
Pharmacokinetics
Quails

ABSTRACT

Pharmacokinetics of difloxacin (DF), a fluoroquinolone antibiotic, were investigated in Japanese quails (Coturnix japonica) after a single intravenous (IV) and oral (PO) administration of 10 mg/kg bodyweight. Plasma concentration profile of DF was analyzed by a compartmental pharmacokinetic method. Following IV injection, the plasma concentration vs time profile was best described by a two-compartment open model. Elimination half-life (t1/2β), total body clearance (Cltot), volume of distribution at steady state (Vdss) and mean residence time (MRT) of DF were 5.45 ± 0.14 h, 0.22 ± 0.01 L/kg/h, 1.54 ± 0.06 L/kg and 6.92 ± 0.19 h, respectively. Following PO administration, DF was rapidly absorbed, with peak plasma concentration (Cmax) of 3.67 μg/mL attained at 1.90 h (Tmax) after administration. Absorption half-life (t1/2ab), elimination half-life (t1/2el), mean absorption time (MAT) were 0.5 h, 5.26 h and 1.11 h, respectively. The bioavailability (F) following PO administration of DF was high (84.40%). For a successful clinical effect of DF in quails, a multiple dosage regimen of 10 mg/kg bodyweight, administered orally every 24 h is recommended to maintain effective plasma concentrations with bacterial infections, in which MIC90 is < 0.2 μg/mL.

1. Introduction

Fluoroquinolones (FQs), synthetic antibacterial agents are widely used clinically because of its excellent antibacterial activity, wide spectrum of activity and higher degree of bioavailability. Japanese quail (Coturnix japonica) can be infected by similar bacterial pathogens that affect chickens. Difloxacin hydrochloride is a synthetic second-generation fluoroquinolone antibacterial agent, act on several kinds of bacteria infecting quails and causing serious problems such as mycoplasma (Kenny et al., 1989), Gram-positive and -negative bacteria (Eliopoulos et al., 1985; Gerchman et al., 2008; Stamm et al., 1986). Difloxacin exhibits a concentration-dependent bactericidal effect by inhibition of bacterial DNA topoisomerase II (gyrase enzyme) which are needed for the transcription and replication of bacterial DNA. As a result DNA replication and transcription are inhibited (Orlica and Zhao, 1997).

The pharmacokinetic parameters of DF have been established in several species as pigs (Inui et al., 1998), non-lactating goats (Atif et al., 2002), lactating goats (Marin et al., 2007a; Marin et al., 2010), sheep (Marin et al., 2007b), dogs (Heinen, 2002), rabbits (Abd El-Aty et al., 2005; Fernandez-varon et al., 2008), horses (Fernandez-Varon et al., 2006), calves (Ismail, 2007), camels (Abo-El-Soud and Goudah, 2009) and chickens (Abo El-Ela et al., 2014; Anadon et al., 2011; Inui et al., 1998). However, there are no any previous data regarding DF pharmacokinetics in quails. Therefore; the current study was carried out to examine the pharmacokinetics of DF in Japanese quails (Coturnix japonica) following single PO and IV administrations.

2. Materials and methods

2.1. Drug and chemical reagents

Difloxacin hydrochloride was kindly supplied by ATCO Pharma for Pharmaceutical Industries, Cairo, Egypt as a pale crystalline powder (99% purity), and reconstituted in distilled water to a final concentration of 3.67 μg/mL attained at 1.90 h (Tmax) after administration. Absorption half-life (t1/2ab), elimination half-life (t1/2el), mean absorption time (MAT) were 0.5 h, 5.26 h and 1.11 h, respectively. The bioavailability (F) following PO administration of DF was high (84.40%). For a successful clinical effect of DF in quails, a multiple dosage regimen of 10 mg/kg bodyweight, administered orally every 24 h is recommended to maintain effective plasma concentrations with bacterial infections, in which MIC90 is < 0.2 μg/mL.

2.2. Experimental birds

All quails were maintained well in accordance with the recommendations of ‘Guide for the Care and Use of Laboratory Animals’ ratified by the Ethical Committee of Benha University. Fifty clinically healthy adult male and female Japanese quails (Coturnix japonica), weighing 188 ± 16.3 g, were utilized to examine the pharmacokinetic...
parameters of DF. Quails were housed in groups (n = 5, in cages) and fed on a commercial drug-free ration and water was provided ad litterum. Quails were kept for two weeks to acclimatize the environment before the experiment began. All quails were physically good before drug administration.

2.3. Experimental design

A sequential design with two weeks wash-out period between the two routes of DF administration was used. No data for the dose of difloxacin were recorded before in quail, so we used 10 mg/kg bwt, which was previously used in chickens (Abo El-Ela et al., 2014; Anadon et al., 2011). Quails were divided into 10 groups (n = 5), DF was administered as a single IV dose into the right brachial vein. After two weeks period, quails have dosed DF at the same dose level directly into the crop using 1-cc, 26 G syringe (Aboubakr, 2012). Each quail was blood-sampled only once (not > 1% of bodyweight). Blood samples from different groups were collected from the left brachial vein at 0.0833, 0.166, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h (n = 5 birds/time-point), into EDTA treated tubes. Plasma samples were separated after centrifugation at 2000g for 10 min, and kept at –20°C until the analysis.

2.4. Analytical method

Difloxacin plasma concentrations were determined by HPLC system as has been done previously (Ding et al., 2008). An aliquot of 200 μL plasma was deproteinized with 200 μL perchloric acid (0.15 M), vigorously vortexed for 2 min and centrifuged at 20,000g for 10 min. The supernatants were aspirated and filtered through a 0.45-μm HPLC filter (Chromatopac®, 4P, Kurabo Biomedical Industries, Ltd., Osaka, Japan) and 50 μL of the filtrate was injected into HPLC system.

The HPLC unit (Shimadzu Corporation, Kyoto, Japan) consisted of a fluorescence detector (RF-10A XL), pump (LC-10AD), loop injector (model 7125) and integrator (Chromatopac C-R7A plus). The mobile phase was a mixture of acetonitrile and 0.0174 M/L tetra-butylammonium bromide solution (95:905, v/v, pH 3.0). Analytical separation was accomplished by a Hypersil BDS C18 column (5 μm, 4.6 × 250 mm). The flow rate was 1 mL/min. and the fluorescence detector was run at an excitation/emission wavelength of 278/465 nm. Samples preparation and analysis were conducted at room temperature.

The calibration was done by spiking 500 μL of blank quail plasma with 20 μL of a series of diluted DF working standard solutions ranged from 0.05 to 20 μg/mL and analyzed as mentioned above. The standard curve of DF was linear with correlation coefficients value of 0.997. Values of LOD and LOQ were 0.05 and 0.1 μg/mL, respectively. The Mean recovery rate of DF from plasma was 99.45%. The intra-day CV values of LOD and LOQ were 0.05 and 0.1 μg/mL, respectively. The standard deviation was 0.0833, 0.166, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h (n = 5 birds/time-point, quails have dosed DF at the same dose level directly into the crop using 1-cc, 26 G syringe (Aboubakr, 2012). Each quail was blood-sampled only once (not > 1% of bodyweight). Blood samples from different groups were collected from the left brachial vein at 0.0833, 0.166, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h (n = 5 birds/time-point), into EDTA treated tubes. Plasma samples were separated after centrifugation at 2000g for 10 min, and kept at –20°C until the analysis.

2.5. Pharmacokinetic analysis

Difloxacin calculated concentrations following IV and PO administrations were subjected to a compartmental and non-compartmental analysis using a computerized program, WinNonlin 4.1 (Pharsight, Mountain View CA, USA). The pharmacokinetic parameters, CLtot, VDss and MRT were estimated (Gibaldi and Perrier, 1982). After PO administration, DF concentrations in quail’s plasma were analyzed by one compartment model. The AUC, UMC were calculated by the method of trapezoids (from time zero to the last sampling time) and integration (from the last sampling time to infinity). The infinite time was calculated with the terminal elimination rate constant, which was detected by the nonlinear least-square iterative technique based on four data points in the terminal portion of the concentration-time curve. The elimination half-life at β phase (t1/2β) was calculated as t1/2β = 0.693/β, where β is the elimination rate constant calculated by the linear regression from the terminal linear section of the plasma concentration vs time curve. MRT = AUMC/AUC and Cltot = Dose/AUC0∞. Bioavailability (F) = AUCPO/AUCIVx100 and MAT = MRTPO - MRTIV.

2.6. Statistical analysis

Results obtained were displayed as (mean ± SE). The paired t-test was used to test the differences between the two administration routes. Statistical software SPSS, version 16 (SPSS Inc., Chicago, USA) was used for statistical analysis and values of P ≤ .05 were considered significant.

3. Results

No side reactions to DF occurred after IV or PO administration. Difloxacin was found to be accurately resolved as a single sharp peak with a retention time of 6–7 min. No interference peak was observed on chromatograms. After IV injection, the plasma concentration vs time data of DF in quails fit well with the two-compartment open model. The mean ± SE plasma concentration vs time profiles of DF after a single IV and PO administration of 10 mg/kg bodyweight in quails were presented graphically in Fig. 1. Mean ± SE values of pharmacokinetics parameters estimated from the curve fitting were shown in Table 1.

4. Discussion

Disposition of FQs has been investigated in several avian species, and inter-species differences in the pharmacokinetic properties have been demonstrated. Enrofloxacin and danofloxacin showed higher volumes of distribution than marbofloxacin in poultry and wild birds (Anadon et al., 2002; de Lucas et al., 2004; Dimitrova et al., 2007; Haritova et al., 2006a). A large differences in values for enrofloxacin clearance and smaller variations for danofloxacin and marbofloxacin, have been also reported in different avian species (Anadon et al., 1995; Carpenter et al., 2006; de Lucas et al., 2004; Flammer et al., 1991; Garcia-Montijano et al., 2011; Garcia-Montijano et al., 2001; Goudah and Mouneir, 2009; Haritova et al., 2006b; Harrenstien et al., 2000). These variances in FQs disposition among avian species necessitate careful evaluation prior its clinical use. Dose extrapolation from other species using allometric scaling could lead to mistakes, particularly when drugs are subjected to alteration in metabolism.

Following IV injection of DF in quails, the disposition kinetic curve declined in a biphasic manner, suggesting that DF disposition obeyed two-compartment open model. This finding is in agreement with DF previous reports in goats (Atef et al., 2002; Marin et al., 2007a), pigs and chickens (Ding et al., 2008; Inui et al., 1998) and agreed with other
Table 1
Mean ± SE plasma pharmacokinetic parameters of DF in Japanese quails following a single IV and PO administration of 10 mg/kg bodyweight (n = 5).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>IV</th>
<th>PO</th>
</tr>
</thead>
<tbody>
<tr>
<td>α (ka0)</td>
<td>h⁻¹</td>
<td>2.23 ± 0.09</td>
<td>1.36 ± 0.06 *</td>
</tr>
<tr>
<td>t₁/₂α (t1/2αa)</td>
<td>h⁻¹</td>
<td>0.29 ± 0.01</td>
<td>0.50 ± 0.02 *</td>
</tr>
<tr>
<td>β (Kd0)</td>
<td>h⁻¹</td>
<td>0.12 ± 0.002</td>
<td>0.13 ± 0.001 t</td>
</tr>
<tr>
<td>t₁/₂β (t1/2β)</td>
<td>h</td>
<td>5.45 ± 0.14</td>
<td>5.26 ± 0.11</td>
</tr>
<tr>
<td>AUC</td>
<td>pg·h/mL</td>
<td>44.9 ± 2.03</td>
<td>38.0 ± 1.85</td>
</tr>
<tr>
<td>AUMC</td>
<td>pg·h/mL</td>
<td>310.4 ± 21.7</td>
<td>304.3 ± 23.8</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>6.92 ± 0.19</td>
<td>8.03 ± 0.23 *</td>
</tr>
<tr>
<td>MAT</td>
<td>h</td>
<td>—</td>
<td>1.11 ± 0.04</td>
</tr>
<tr>
<td>tmax</td>
<td>h</td>
<td>—</td>
<td>1.90 ± 0.03</td>
</tr>
<tr>
<td>Vdss</td>
<td>L/kg</td>
<td>1.54 ± 0.06</td>
<td>—</td>
</tr>
<tr>
<td>Cltot</td>
<td>L/h/kg</td>
<td>0.22 ± 0.01</td>
<td>—</td>
</tr>
<tr>
<td>Cmax</td>
<td>µg/mL</td>
<td>—</td>
<td>3.67 ± 0.12</td>
</tr>
<tr>
<td>F%</td>
<td>—</td>
<td>—</td>
<td>84.4 ± 5.41</td>
</tr>
</tbody>
</table>

α, β = hybrid rate constant representing the slope of distribution and elimination phase after IV injection; Ka0, Kd0 = absorption and elimination rate constant after PO administration; t₁/₂α = distribution half-life after IV injection; t₁/₂a = absorption half-life after PO administration; t₁/₂β = elimination half-life after IV injection; t₁/₂β = elimination half-life after PO administration; AUC = area under plasma concentration-time curve; AUMC = area under moment curve; MRT = mean residence time; MAT = mean absorption time; Vd0 = volume of distribution at steady state; tmax = total body clearance.

Cmax = maximum plasma concentration; tmax = time to peak concentration; F = fraction of drug absorbed systemically after oral administration.

* Significant P ≤ .05. — Not calculated.

DFoxacin has good distribution characteristics as shown by a short distribution half-life (1/2a = 0.29 h). Similar findings have been recorded for other FQs as doxycyclin in ducks (0.30 h, Aboubakr and Soliman, 2014) and shorter than DF in broiler chicken (0.69 h, Ding et al., 2008; 0.66 h, Inui et al., 1998). The Vd0 for DF in the current study was 1.54 L/kg suggesting a wide distribution of DF in tissues of quails after IV administration. Higher values for the volume of distribution of other FQs in healthy Japanese quail after IV injection were recorded. The Vd0 of enrofloxacin (10 mg/kg), danofloxacin (10 mg/kg), and marbofloxacin (5 mg/kg) were 5.36, 6.87 and 1.25 L/kg, respectively, suggesting that the highest concentrations of these drugs were found in quail’s plasma (Hartova et al., 2013).

Our results showed that, elimination half-life (t1/2β) of DF in Japanese quails was (5.45 h), longer than those of levofloxacin in turkeys (4.49 h, Aboubakr et al., 2014)) and quails (2.52 h, Aboubakr, 2012)) and enrofloxacin, danofloxacin, and marbofloxacin in quails (2.45, 3.66 and 2.1 h, respectively, (Hartova et al., 2013)).

In the current study, the total body clearance of DF was (0.22 L/h/kg) suggesting a relatively faster clearance rate of DF in quails. Nearly equal value has been detected for DF in horses (0.28 L/h/kg, Fernandez-Varon et al., 2006). Contrarily, a higher clearance value of DF was recorded in broilers (0.72 L/h/kg, Inui et al., 1998) and 0.37 L/h/kg, (Ding et al., 2008)). Higher clearance values in Japanese quail after IV injection of enrofloxacin (10 mg/kg), danofloxacin (10 mg/kg), and marbofloxacin (5 mg/kg body weight) were 1.52, 1.64 and 0.43 L/h/kg, respectively (Haritova et al., 2013). In Japanese quails, rapid clearance of orfloxacin (0.60 L/h/kg, (Hawkings et al., 2011)) and moxifloxcin (0.41 L/h/kg, (Goudah and Hasabelnaby, 2016)) were also reported.

Following PO administration, DF was rapidly and efficiently absorbed through the gastrointestinal tract of quails as the absorption half-life (t1/2a) was 0.5 h. This value being reasonably similar to that reported for orfloxacin in quails (0.59 h, (Hawkings et al., 2011)), longer than marbofloxacin in Muscovy ducks (0.36 h, (Goudah and Hasabelnaby, 2011)) and shorter than data reported in quails for both levofloxacin (1.07 h, (Aboubakr, 2012)) and moxifloxacin (0.91 h, (Goudah and Hasabelnaby, 2014)). The rapid oral absorption of DF was also reflected by low MAT (1.11 h) and this value was nearly similar to that of enrofloxacin (1.20 h) in chickens (Knoll et al., 1999). The elimination half-life (t1/2β) of DF in quails was 5.26 h, which was longer than moxifloxacin in quails (1.79 h, (Goudah and Hasabelnaby, 2014)) and shorter than marbofloxacin in chickens (8.69 h, (Anadon et al., 2002)).

The peak plasma concentrations (Cmax) following PO administration of DF in quails were 3.67 µg/mL, nearly similar to that reported for ofloxacin (10 mg/kg in chickens (3.65 µg/mL, (Kalaiselvi et al., 2006)) and lower than orfloxacin (10 mg/kg) in quails (5.22 µg/mL, (Hawkings et al., 2011)). The absorption process was rapid with time to reach maximum concentration (Cmax) of 1.90 h, which was similar to moxifloxacin in quails (1.87 h, (Goudah and Hasabelnaby, 2014)).

The systemic bioavailability of DF following PO administration was (84.4%) which was almost the same as oral bioavailability in quails reported for danofloxacin (88.08%) and moxifloxacin (87.9%) by (Goudah and Hasabelnaby, 2014; Haritova et al., 2013), respectively, and higher than that of marbofloxacin (56.3%, (Haritova et al., 2013)) and lower than orfloxacin (102.01%, (Hawkings et al., 2011)).

For predicting the efficacy of a concentration dependent-anti-bacterial agents, using the surrogate marker AUC24/MIC above 100 (Lode et al., 1998) and Cmax/MIC above 8 (Madaras-Kelly et al., 1996), DF would have success against microorganisms in quails with MIC ≤ 0.20 µg/mL after PO dosing. DF administered orally at a dose of 10 mg/kg bodyweight every 24 h in Japanese quails would be efficacious against many bacterial diseases affecting quails as chronic respiratory infections caused by sensitive strains of Escherichia coli and Mycoplasma gallisepticum, avian choleras caused by Pasteurella multocida and colibacillosis.

5. Conclusions
Administration of DF (10 mg/kg bodyweight) might be highly efficacious against susceptible bacteria in quails. Further studies on tissue residues of DF are required.

Declarations of interest
None.

References
Garcia-Martinez, J.D., 2008. Disposition kinetics and pharmacokinetics—pharmacody-
namic integration of di-oxacin against Staphylococcus aureus isolates from rab-

Gerchman, I., Lysnyansky, I., Perk, S., Levisohn, S., 2002. Comparative pharmacokinetics of en-
rofloxacin after a long-acting formulation in young domestic ostrich (<i>Struthio camelus</i>). J. Vet.
Pharmacol. Ther. 27, 119–122.

Georges-Kelly, K.J., Ostergaard, B.E., Hovde, L.B., Rotschafer, J.C., 1996. Twenty-four-
hour pharmacokinetics of the fluoroquinolones enro-
fl oxacin, dano-fl oxacin, marbo-fl oxacin and orbifl oxacin in dogs after single oral ad-


Gamboa, F., Martinez-Larranaga, M.R., 2011. Plasma disposition and tissue depletion of di-
foxacin and its metabolite sarafloxacin in the food producing animals, chickens

Godinho, G., Monteiro, M., Lopes, A., Amaro, R., 2007. Pharmacokinetics of en-

Goudah, A., Aboubakr, M., Elbadawy, M., 2009. Disposition kinetics and tissue residues of dano-

Goudah, A., Hasabelnaby, S., 2014. Plasma disposition and tissue residue of Moxi-

Haritova, A., Dimitrova, D., Velcheva, R., 2009. Pharmacokinetics and tissue residues of di-
roxacin in Great Horned Owls (<i>Bubo virginianus</i>) and Eurasian buzzards (<i>Buteo buteo</i>) after intra


rofloxacin in pigs and broilers following intravenous, intramuscular, and oral single-


Agents Chemother. 28, 514–520.

Heinen, E., 2002. Comparative serum pharmacokinetics of the fluoroquinolones enro-
fl oxacin, dano-fl oxacin, marbo-fl oxacin and orbifl oxacin after single oral ad-


Bioavailabilities of Difloxacin in Pig and Chicken. Xenobiotica; the Fate of Foreign

Bioavailabilities of Difloxacin in Pig and Chicken. Xenobiotica; the Fate of Foreign

Iturrizaga, A., Prado, Y., Fernandez-Camacho, P., 2007. Pharmacokinetics and tissue resid-
ues of doxycycline and oxytetracycline in young domestic ostrich (<i>Struthio camelus</i>). J. Vet.

Ishii, Y., Izumi, K., Shimizu, Y., Itoh, S.-I., 2009. Comparative pharmacokinetics of enro-


Bioavailabilities of Difloxacin in Pig and Chicken. Xenobiotica; the Fate of Foreign


Kroll, N., Gluder, G., Kiehm, M., 1999. Comparative study of the plasma pharma-
 kokinetische und tissue concentrations of dano-fl oxacin and enrofl oxacin in broiler

Kroll, N., Gluder, G., Kiehm, M., 1999. Comparative study of the plasma pharma-
 kokinetische und tissue concentrations of dano-fl oxacin and enrofl oxacin in broiler

Kroll, N., Gluder, G., Kiehm, M., 1999. Comparative study of the plasma pharma-
 kine und tissue concentrations of dano-fl oxacin and enrofl oxacin in broiler

Kroll, N., Gluder, G., Kiehm, M., 1999. Comparative study of the plasma pharma-
 kine und tissue concentrations of dano-fl oxacin and enrofl oxacin in broiler

Kroll, N., Gluder, G., Kiehm, M., 1999. Comparative study of the plasma pharma-
 kine und tissue concentrations of dano-fl oxacin and enrofl oxacin in broiler

Kroll, N., Gluder, G., Kiehm, M., 1999. Comparative study of the plasma pharma-
 kine und tissue concentrations of dano-fl oxacin and enrofl oxacin in broiler

Kroll, N., Gluder, G., Kiehm, M., 1999. Comparative study of the plasma pharma-
 kine und tissue concentrations of dano-fl oxacin and enrofl oxacin in broiler