PHARMACOKINETICS AND TISSUE RESIDUES OF NORFLOXACIN IN NORMAL AND EXPERIMENTALLY *E.Coli* INFECTED BROILER CHICKEN.


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**ABSTRACT**

The pharmacokinetics of norfloxacin was studied following single intravenous, oral administration in normal chicken and repeated oral administrations of norfloxacin in normal and experimentally *E.coli* infected broiler chicken. The pharmacokinetic parameters following a single intravenous injection of 10 mg/kg b.wt., revealed that norfloxacin obeyed a two compartments open model, distribution half-life ($t_{0.5(\alpha)}$) was 0.149±0.001 h, volume of distribution ($V_{dss}$) was 2223.17±65.62 ml/kg, elimination half-life ($t_{0.5(\beta)}$) was 4.71±0.06 h and total body clearance ($CL_{tot}$) was 1.72±0.02 ml/kg/min. Following oral administration, norfloxacin was rapidly and efficiently absorbed through gastrointestinal tract of chicken as the absorption half-life ($t_{0.5(ab)}$: 0.57±0.01 h). Maximum serum concentration ($C_{max}$) was 3.87±0.04 µg/ml, reached its maximum time ($t_{max}$) at about 2.09±0.03 h, elimination half-life ($t_{0.5(\beta)}$) was 5.72±0.05 h and total body clearance ($CL_{tot}$) was 4.02±0.06 ml/kg/min indicating the tendency of chicken to eliminate norfloxacin in slow rate. Oral bioavailability was 66.89±1.95 % indicating moderate absorption of norfloxacin after oral administration from oral site. The *in vitro* protein binding was 10.37±0.22 %. Serum concentrations of norfloxacin following repeated oral administration of 10 mg/kg BW once daily for five consecutive days, peaked 2 hours after each oral dose with lower significant values recorded in experimentally infected chicken than in normal ones. Tissues residues of norfloxacin in slaughtered normal and experimentally *E.coli* infected chicken could not be detected by microbiological assay in all tested tissues except in lung, liver, and kidneys in chicken at 48 hours post last administration, so the chicken must not be slaughtered before 3 days of stopping of drug administration.

**Key words:** Norfloxacin, Pharmacokinetics, Tissue residues, Chicken.

1. INTRODUCTION

Norfloxacin is a synthetic antibacterial belonging to the fluoroquinolones class and is usually prescribed for the treatment of urinary tract infections. Fluoroquinolones are gaining popularity as important antibacterial agents in veterinary practice because of their broad antimicrobial activity (Park *et al.*, 1998). Quinolones are active against gram negative and gram-positive bacteria *in vitro* (Wolffson and Hooper, 1985), as well as trimethoprim/sulfonamide resistant microbes (Preheim *et al.*, 1987). In addition, these antimicrobials are also active against Mycoplasma (Brown, 1996). Moreover, no plasmid resistance has been demonstrated and fluoroquinolones have a favorable margin of safety (Bahri and Blouin, 1991). The pharmacokinetics of norfloxacin in laboratory animals (Gilfillan *et al.*, 1984), dogs (Walker *et al.*, 1989; Brown *et al.*, 1990), chicken (Anadon *et al.*, 1992), pig (Anadon *et al.*, 1995), donkeys (Lavy *et al.*, 1995), calves (Gips and Soback, 1984), sheep (Gonzalez *et al.*, 1986), and sheep (Yassin and El-Ashry, 1985) have been studied.
1997), cow (Shem-Tov et al., 1998), horse (Park and Yun, 2003), rabbits (Pavithra et al., 2009) and goats (El-Sayed et al., 2011) was studied.

The aim of this study is to investigate the pharmacokinetic profile of norfloxacin (10 mg/kg b.wt.) following single intravenous, oral administration in normal chicken and repeated oral administrations in normal and experimentally E.coli infected broiler chicken. Also, tissue residues following repeated oral administrations in normal and experimentally E.coli infected broiler chicken was evaluated.

2. MATERIALS AND METHODS

2.1. Drug:
Norfloxacin was obtained as an oral solution from ATCO Pharma for pharmaceutical industries under a trade name (Atonor®). Each ml contains 300 mg of norfloxacin base.

2.2. Experimental chicken:
Forty eight clinically normal Cobb chicken of four weeks of age weighting about 1100 to 1300 gm. Each chosen randomly from Gharbia poultry farm to be used in this investigation. Chicken were fed on a balanced ration free from antibiotics for two weeks to withdraw any antibiotic residues. Before drug administration, the weight of chicken ranged between 1650 to 1850 gm for each.

Grouping of chicken:

Group (1): Six normal chicken were intravenously administrated into the wing vein with a single dose of 10 mg/kg body weight. These chicken were left for 15 days to ensure complete elimination of tested drug from their bodies and then given the same dose by oral administration, to determine the oral bioavailability.

Group (2): Six normal chicken were orally administrated 10 mg/kg b.wt., once daily for five consecutive days, to determine the blood concentrations and pharmacokinetics of the drug.

Group (3): Six experimentally E.coli infected chicken were orally administrated 10 mg/kg b.wt., once daily for five consecutive days, to determine the blood concentrations and pharmacokinetics of the drug.

Group (4): Fifteen normal chicken were orally administrated 10 mg/kg b.wt., once daily for five consecutive days, to determine the blood and tissue residues.

Group (5): Fifteen experimentally E.coli infected chicken were orally administrated 10 mg/kg b.wt., once daily for five consecutive days, to determine the blood and tissue residues.

2.3. Samples:

2.3.1. Blood samples:
About half milliliter of blood was taken from the right wing vein, following administration of the drug. Blood samples were collected at 5, 10, 15, 30 minutes, 1, 2, 4, 8, 12, 24 hours after single intravenous and single oral administration. Blood samples following repeated oral administrations in normal chicken and experimentally infected chicken for 5 consecutive days were taken daily at 15, 30 minutes, 1, 2, 4, 8, 12, 24 hours. All blood samples were collected in sterilized centrifuged tubes and allowed to clot. Serum was separated by centrifugation at 3000 r.p.m for 10 minutes. Sera were kept frozen until assayed.

2.3.2. Tissue samples:
After the end of fifth day of repeated oral administration of norfloxacin, three chicken were slaughtered from group (4) and group (5) at 24, 48, 72, 96, 120 hours. From each slaughtered chicken, lung, heart, liver, kidneys, skin with fat, breast muscles and thigh muscles were taken for drug assay.
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Analytical procedures:
Norfloxacin in both collected blood and tissue samples were assayed using microbiological assay method using E.coli (ATCC 25922) as a test organism for norfloxacin (Arret et al., 1971).
The data were expressed as (Mean ± SE) and analyzed using SPSS (16) software (SPSS Inc., Chicago, USA) and differences between the averages were examined by Duncan’s multiple-range test. Mean values within a row with different superscript letters are significantly different ($P<0.05$).

3. RESULTS

Following a single intravenous injection of 10 mg/kg b.wt. of norfloxacin in normal chicken, norfloxacin could be detected therapeutically for 24 hours. The serum concentration – time curve of norfloxacin following intravenous injection showed that the drug obeyed a two compartments open model. The disposition kinetics of norfloxacin following a single intravenous and oral administration were recorded in table (1) and showed in figure (1). Oral administration of 10 mg/kg.b.wt every 24 hours for five doses in normal and E.coli infected chicken revealed a lower significant serum norfloxacin concentration at all-time sampling in E.coli infected chicken than in normal ones. The pharmacokinetic parameters of norfloxacin after repeated oral administration in normal chicken were compared to those in E.coli as shown in table (2). Tissues residues of norfloxacin in slaughtered normal and E.coli infected chicken could not be detected by microbiological assay in all tested tissues except in lung, liver, and kidneys in chicken at 48 hours post last administration.

Table (1): Pharmacokinetic parameters of norfloxacin in chicken following single intravenous and oral administration of 10 mg/kg b.wt. (n=6)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Intravenous (X ± S.E.)</th>
<th>Oral (X ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_0$</td>
<td>μg ml$^{-1}$</td>
<td>14.86 ± 0.28</td>
<td>—</td>
</tr>
<tr>
<td>$\alpha$ ($k_{ab}$)</td>
<td>h$^{-1}$</td>
<td>4.65 ± 0.03</td>
<td>1.21 ± 0.02</td>
</tr>
<tr>
<td>$\beta$ ($k_{el}$)</td>
<td>h$^{-1}$</td>
<td>0.15 ± 0.002</td>
<td>0.12 ± 0.001</td>
</tr>
<tr>
<td>$t_{0.5a}$ ($t_{0.5ab}$)</td>
<td>h</td>
<td>0.15 ± 0.001</td>
<td>0.57 ± 0.01</td>
</tr>
<tr>
<td>$t_{0.5i}$ ($t_{0.5i}$)</td>
<td>h</td>
<td>5.72 ± 0.05</td>
<td>4.71 ± 0.06</td>
</tr>
<tr>
<td>AUC</td>
<td>μg ml$^{-1}$h$^{-1}$</td>
<td>5.77 ± 0.07</td>
<td>3.86 ± 0.10</td>
</tr>
<tr>
<td>$V_dss$</td>
<td>l kg$^{-1}$</td>
<td>2.22 ± 0.07</td>
<td>—</td>
</tr>
<tr>
<td>$Cl$</td>
<td>ml kg$^{-1}$m$^{-1}$</td>
<td>1.72 ± 0.02</td>
<td>4.02 ± 0.06</td>
</tr>
<tr>
<td>$C_{max}$</td>
<td>μg ml$^{-1}$</td>
<td>—</td>
<td>3.87 ± 0.04</td>
</tr>
<tr>
<td>$t_{max}$</td>
<td>h</td>
<td>—</td>
<td>2.09 ± 0.03</td>
</tr>
<tr>
<td>F</td>
<td>%</td>
<td>—</td>
<td>66.89 ± 1.95</td>
</tr>
</tbody>
</table>
Table (2): Pharmacokinetic parameters of norfloxacin in normal (N) and experimentally *E. coli* infected chicken (E) during repeated oral administration of 10 mg/kg b.wt., once daily for five consecutive days (n=6).

<table>
<thead>
<tr>
<th>Day</th>
<th>Parameter</th>
<th>Unit</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>µg/ml</td>
<td>5.78 ± 0.11</td>
<td>4.66 ± 0.08</td>
<td>8.77 ± 0.06</td>
<td>7.63 ± 0.23</td>
<td>5.11 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>K_{ab}</td>
<td>h⁻¹</td>
<td>1.21 ± 0.02</td>
<td>1.33 ± 0.01</td>
<td>1.18 ± 0.01</td>
<td>1.36 ± 0.01</td>
<td>1.53 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>t_{0.5ab}</td>
<td>H</td>
<td>0.56 ± 0.56</td>
<td>0.53 ± 0.56</td>
<td>0.64 ± 0.56</td>
<td>0.51 ± 0.46</td>
<td>0.46 ± 0.50</td>
</tr>
<tr>
<td></td>
<td>C_{max}</td>
<td>µg/ml</td>
<td>4.01 ± 0.002</td>
<td>3.56 ± 0.003</td>
<td>4.67 ± 0.003</td>
<td>4.78 ± 0.003</td>
<td>7.00 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>C_{min}</td>
<td>µg/ml</td>
<td>0.31 ± 0.01</td>
<td>0.27 ± 0.002</td>
<td>0.39 ± 0.01</td>
<td>0.41 ± 0.01</td>
<td>0.71 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>C_{max}</td>
<td>µg/ml</td>
<td>4.09 ± 0.003</td>
<td>3.27 ± 0.01</td>
<td>4.96 ± 0.04</td>
<td>4.58 ± 0.05</td>
<td>7.53 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>t_{max}</td>
<td>H</td>
<td>2.06 ± 0.06</td>
<td>2.67 ± 0.04</td>
<td>2.67 ± 0.04</td>
<td>2.09 ± 0.05</td>
<td>2.09 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>µg/ml</td>
<td>4.67 ± 0.08</td>
<td>3.89 ± 0.04</td>
<td>7.34 ± 0.03</td>
<td>5.39 ± 0.02</td>
<td>8.97 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>K_{el}</td>
<td>h⁻¹</td>
<td>0.123 ± 0.002</td>
<td>0.117 ± 0.002</td>
<td>0.122 ± 0.001</td>
<td>0.110 ± 0.001</td>
<td>0.079 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>t_{0.5b}</td>
<td>H</td>
<td>5.57 ± 0.14</td>
<td>5.95 ± 0.08</td>
<td>5.75 ± 0.06</td>
<td>6.32 ± 0.06</td>
<td>8.72 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>CL_{tot}</td>
<td>ml/kg/min</td>
<td>0.11 ± 0.02</td>
<td>0.02 ± 0.02</td>
<td>0.02 ± 0.03</td>
<td>0.03 ± 0.03</td>
<td>0.04 ± 0.04</td>
</tr>
</tbody>
</table>

Mean values having different letters in column differ significantly (*P*<0.05).
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4. DISCUSSION

In the present investigation, intravenous injection of 10 mg/kg b.wt. of norfloxacin in normal chicken, showed that the drug disposition best fitted a two-compartments-open model, a compartment of plasma and rapid equilibrating tissues, and a deeper slower compartment. The obtained result was consistent with those reported for norfloxacin in dogs (Brown et al., 1990), broiler chicken (Anadon et al., 1992), donkeys (Lavy et al., 1995), rabbits (Park et al., 1995) and horses (Park and Yun, 2003). Norfloxacin was eliminated in current study following a single intravenous injection with elimination half-life ($t_{0.5(\text{el})}$) = 4.71±0.06 h. This observation agreed with the data reported after intravenous injection of norfloxacin (5.44 h) in horses (Park and Yun, 2003). On contrast this value was longer than those recorded in other species as (3.56 h) in dogs (Brown et al., 1990), (2.1 h) in swine (Shem-Tov et al., 1994), (3.65 h) in pigs (Anadon et al., 1995), (3.51 h) in donkeys (Lavy et al., 1995) and (3.93 h) in rabbits (Park et al., 1998). On the other hand, it was shorter than (8 h) in chicken (Anadon et al., 1992) and (7.42 h) in pigs (Chang et al., 2007). Such differences are relatively common and frequently related to interspecies variation, assay methods used, the time between blood samplings, and / or the health status and age of the animals (Haddad et al., 1985). The $V_{ds}$ for norfloxacin was 2.22 l/kg, suggesting good penetration through biological membranes and tissue distribution after intravenous administration in broiler chicken. The obtained value was longer to that recorded for marbofloxacin (1.41 l/kg) in turkeys (Haritova et al., 2006a), marbofloxacin (0.57 l/kg) in muscovy ducks (Goudah and Hasabelnaby, 2011) and shorter than danofloxacin and enrofloxacin (6.59, 3.57 l/kg) in turkeys (Haritova et al., 2006b; Dimitrova et al., 2007), respectively. The total body clearance ($CL_{tot}$) was 0.10 l/h/kg, this value was close to other fluoroquinolones as marbofloxacin (0.23 l/h/kg) in muscovy ducks (Yuan et al., 2011), but shorter than danofloxacin (0.59 l/h/kg) in turkeys (Haritova et al., 2006).
Following oral administration, norfloxacin was rapidly and efficiently absorbed through gastrointestinal tract of broiler chicken as the absorption half-life \((t_{0.5(ab)})\) was 0.57±0.01 h. The obtained value was longer than marbofloxacin (0.36 h) in muscovy ducks (Yuan et al., 2011) and shorter than difloxacin (1.74 h) in chickens (Anadon et al., 2011). The elimination half-life \((t_{0.5(el)})\) was 5.72±0.05 h. This value was nearly similar to marbofloxacin (4.61 h) in muscovy ducks (Yuan et al., 2011) and shorter than those for norfloxacin, marbofloxacin, danofloxacin and enrofloxacin (9.07, 9.74, 7.73, 6.92 h, respectively) in turkeys (Laczay et al., 1998; Haritova et al., 2006a; Haritova et al., 2006b; Dimitova et al., 2007 ).

Maximal plasma concentration \((C_{max})\) was 3.87±0.04 μg/ml achieved at \((t_{max})\) 2.09±0.03 h. These values were similar to difloxacin (4.34 μg/ml) in chickens (Ding et al., 2008). The \((C_{max})\) obtained in present study was higher than those reported in healthy broiler chicken (1.96 μg/ml), turkeys (0.95 μg/ml) and geese (1.58 μg/ml) given norfloxacin at a dose level of 10 mg/kg BW (Laczay et al., 1998).

The bioavailability of norfloxacin in normal broiler chicken was 66.89±1.95 %. This value referred to a better absorption of norfloxacin from gastrointestinal tract. This value was nearly similar to those recorded for norfloxacin (73.51 %) in lambs (Gonzalez et al., 1997), danofloxacin (65.70 %) in goats (Atef et al., 2001) and pefloxacin (70.63 %) in lactating goats (Abd El-Aty and Goudah, 2002). On other hand, this value was higher than the bioavailabilities recorded for norfloxacin (35-46 %) in dogs (Brown et al., 1990), (31.5 %) donkeys (Lavy et al., 1995) and (45 %) in rabbits (Park et al., 1998). Also these values were lower than the bioavailability recorded for ciprofloxacin (95.92 %) in lactating goats (El-Banna and Abo-El-Sooud, 1998). In vitro plasma protein binding showed that, norfloxacin displayed a low level of binding to plasma proteins (10.37±0.22 %) to broiler chicken plasma. The results of in vitro protein binding may differ substantially depending on the methodology and experimental conditions (Zlotos et al., 1998). This value was lower than those reported values of 27% for danofloxacin in turkey (Haritova et al., 2006b) and 23.52% for levofloxacin in quails (Aboubakr, 2012).

The obtained blood levels of norfloxacin in Escherichia coli infected broiler chicken were significantly lower than those in normal chicken following repeated oral administrations. These lower blood concentrations in infected chicken might be attributed to the higher penetrating power of norfloxacin to the diseased tissues (Baggot, 1980). The relative higher plasma concentrations of norfloxacin after the last dose compared to the first doses indicating the accumulation of norfloxacin in blood during multiple dosing at 24 hours intervals for five consecutive days. These observations agreed with the progressive daily increase in the mean serum concentrations following the intramuscular injection of ciprofloxacin in lactating goats in a daily dose of 5 mg/kg body weight for five consecutive days (El-Banna and Abo-El-Sooud, 1998).

Following repeated oral administration of 10 mg/kg b.wt. of norfloxacin once daily in normal and experimentally infected chicken for five consecutive days, the drug could not be detected by microbiological assay in all tested tissues except in lung, liver, and kidneys at 48 hours post last administration. In particular the high clearance of norfloxacin indicated the reduced possibility of finding residues of antimicrobial in broiler chicken a few days after treatment and necessity of shorter withdrawal time for this antimicrobial i.e. three days. The obtained results was shorter than that recorded after oral administration of moxifloxacin at 5 mg/kg.
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for 5 days, a pre-slaughter withdrawal time of more than 7 days is needed to ensure that the drug is eliminated from the tissues (Goudah, 2009).

It could be concluded that oral administration of norfloxacin at 10 mg/kg b.wt. may be highly efficacious against susceptible bacteria in broiler chicken. Chicken must not be slaughtered before 3 days of stopping norfloxacin administration.

5. REFERENCES

coli infected lactating goats. IJAVMS. 5: 123-137.
Walker, R.D., Stein, G.E., Budsberg, S.C., MacDonald, K.H. 1989. Serum and tissue fluid norfloxacin concentrations after oral administration of the drug to
حركة ومتغيرات الأنسجة للنورفلوكساسين في الدجاج السليم والمصاب تجريبياً

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المتخصصة في

اجريت هذه الدراسة على عدد 48 من دجاج التسمين (27 سلالة و21 مصاصة بميكروب الفولون العمومي) وقد تم تقسيم الدجاج إلى 5 مجموعات وذلك لدراسة حركة 10 مجم/ كجم من وزن الجسم من عقار النورفلوكساسين عند إعطانية مرة واحدة عن طريق الوريد أو عن طريق الفم وعدد تناوله بالفم واحدة يوميا لمدة خمسة أيام متتالية، كم تم قياس معدل خروج الدواء و معدل الأنخة و قيادة الدواء في نسج الدجاج السليم وكذلك المصاب. كما تم أيضا قياس نسبة إمداد عقار النورفلوكساسين بروتين الدم عموميا. وقد وجد أنه عند حقن النورفلوكساسين في الوريد مرة واحدة بجرعة 10 مجم/ كجم من وزن الجسم في الدجاج السليم أمكن قياس تركيز الدواء في مصل الدجاج لمدة 24 ساعة بعد الحقن. حيث وصل تركيز النورفلوكساسين في نهاية الـ 24 ساعة إلى 0.29 ± 0.09 ميكروجرام/ملي. وقد وجد أنه بعد تناول عقار النورفلوكساسين بالفم مرة واحدة بجرعة 10 مجم/ كجم من وزن الجسم وصل أعلى تركيز للدواء في الدم بعد ساعتين وهو 3.87 ± 0.04 ميكروجرام/ مللي وليست وجد الدواء في الدم حتى الـ 24 ساعة من تناوله ليصل تركيزه إلى 0.28 ± 0.01 ميكروجرام/ مليلي. وكم معدل الأنخة للدجاج السليم بعد تناول عقار النورفلوكساسين بالفم هي 66.89 ± 1.95% وهذه النسبة تعبر عن احتمال مستمر لعلاج النورفلوكساسين بعد تناوله بالفم. وقد وجد عمليا أن نسبة إمداد النورفلوكساسين مع بروتين الدم كانت 10.37 ± 0.22%. وقد وجد أن النورفلوكساسين بالفم مرة واحدة يوميا لمدة أربع أيام متتالية بجرعة 10 مجم/ كجم من وزن الجسم في الدجاج السليم وكذلك الدجاج المصاب، تم قياس معدل ميكروب الفولون العمومي أن هناك زيادة معتدلة في تركيز الدواء في مصل الدجاج السليم بعد تناوله. ويدرس عينة من صابة فولون السليم بعد إجراء الفحص. وكما وجد ان تركيز النورفلوكساسين في الدم بعد تناوله في الدجاج السليم مع ذالك الدجاج المصاب بعد إجراء الفحص، وجد أن تركيز النورفلوكساسين لم يمكن قياسه بطريقة المعايرة الميكروبية في جميع الأنسجة التي تم فحصها

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