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Comparative studies on the pharmacokinetics of norfloxacin in chickens, turkeys and geese after a single oral administration

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Norfloxacin, (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[1-piperazinyl]-3-quinoline carboxylic acid) is a fluoroquinolone antibacterial agent with a high antimicrobial activity against a very wide range of gram-negative and a number of gram-positive aerobes as well as most pathogenic mycoplasmas (Ito *et al.*, 1980; King *et al.*, 1982; Norrby & Jonsson, 1983; Hannan *et al.*, 1989). It is rapidly bactericidal and also active against organisms that may be resistant to other antibacterials such as sulphonamides, β-lactam antibiotics, tetracyclines, aminoglycosides and macrolides (Neu & Labthavikul, 1982; Crumplin *et al.*, 1984). The favourable antimicrobial properties of norfloxacin indicate that it might have great potential for treating common infections such as mycoplasmosis, colibacillosis and pasteurellosis in chickens, turkeys and geese. Publications concerning pharmacokinetic studies of norfloxacin in birds are scarce (Anadón *et al.*, 1992; Gulkaroc & Ziv, 1994; Ramadan *et al.*, 1994), and are mainly focused on the kinetic features and residues of the drug in chickens. The present studies were undertaken to obtain comparative pharmacokinetic data after a single oral administration of a commercial norfloxacin product in chickens, turkeys and geese.

Six healthy male animals 7–8 weeks of age were used in each animal species tested. Commercial broiler chickens (Arbor Acres), turkeys (BIG 6) and domestic white geese were purchased from a poultry farm and housed in individual cages in an experimental animal house. The body weights (mean ± SD) of chickens, turkeys and geese were 1661.7 ± 109.6, 2246.7 ± 135.6 and 3920 ± 144.6 g, respectively. Before the commencement of the experiment the animals were acclimatized for 2 weeks. The room temperature ranged between 20–22°C and the relative humidity was maintained at 50–70%. Commercial diets and water were provided *ad libitum*. The rations did not contain any drug or growth promoter. The birds of each species were treated orally with the commercial product Vetriflox 20% Oral Solution (Lavet Ltd., Budapest, Hungary). This oral formulation containing norfloxacin (200 mg/mL) and stabilizing excipients in 10% acetic acid is an authorized product (Reg. No 655/1996) for use in domestic animals in Hungary. One millilitre of the drug product was diluted with distilled water to a total volume of 40 mL and 2 mL of this solution was given directly into the crop for each kg of body weight. Thus, the dose of norfloxacin was 10 mg/kg. Food was withheld for 12 h before dosing until 6 h after drug administration. From each animal, blood samples were taken via cannula from the brachial vein into syringes at 0 (pre-dose), 0.17, 0.33, 0.50, 0.67, 1, 2, 3, 4, 6, 8, 12 and 24 h after dosing. Plasma samples were separated after centrifugation at 5°C and 2000 ***g*** for 10 min and were stored frozen at −24°C until analysed.

Plasma norfloxacin concentrations were determined by reverse phase high-performance liquid chromatography (HPLC) with fluorescence detection. The applied method was based on those previously described by Anadón *et al.* (1992), Forchetti *et al.* (1984) and Nilsson-Ehle (1987). Briefly, the HPLC system was composed of a 510 pump (Waters, Milford, MA, USA), a Reodyne 7125 injector (Cotati, Redwood, CA, USA), a 745B integrator (Waters) and a HP1046A fluorescence detector (Hewlett Packard, Waldbron, Germany). A Lichrosorb RP18 column (C_{18}, 5 mm, 200 × 4.6 mm; Hewlett Packard) was used for chromatographic separation. The mobile phase consisted of a mixture of acetonitril and bidistilled water containing 4.54 g/L of KH_2PO_4, 5.94 g/L of Na_2HP_4 and 1.94 g/L of (n-C_4H_9) N^+HSO_4- (10:90 v/v), pH 3.0, and flow rate was 1.0 mL/min. Fluorescence detection was performed by excitation at 280 nm and by monitoring the emission at a wavelength of 445 nm. The assay procedure was as follows: to 0.25 mL of plasma 0.25 mL of 0.5 M sodium phosphate buffer (pH 7.5) and 10 mL methylene chloride were added. After shaking and centrifugation (2500 ***g***, 10 min) the separated organic phase was evaporated under a nitrogen stream at 55°C. The residue was redissolved in 0.5 mL of mobile phase, centrifuged (2200 ***g***, 10 min) and 50 mL was injected into the HPLC system. Norfloxacin plasma concentrations were quantified against calibration curves of plasma samples spiked with norfloxacin reference standard (Sigma, St. Louis, MO, USA).

The quantification limit was 0.002 mg/L and the standard curves were linear within the range of 0.002 and 2 mg/mL for plasma of chickens, turkeys and geese, respectively. The recovery rates were greater than 80% for plasma of each animal species tested. The intra- and inter-assay coefficients of variation at five different concentrations (0.002, 0.01, 0.1, 0.5 and 2 mg/mL) were less than 10%. The method used was selective for the

compound analysed; endogenous interference was not observed on chromatograms.

The plasma concentration–time data for each individual bird were analysed by one-compartment and two-compartment open models using the MedUsa (Version 1.6) computer program (Várkonyi, 1983). The area under the plasma concentration vs. time curve (*AUC*) was obtained from the experimental data using the trapezoidal rule and was extrapolated to infinity by dividing the last plasma concentration by the terminal elimination rate constant. The other calculated kinetic parameters were: first-order rate constant for appearance of norfloxacin in the blood (k_a), hybrid rate constants for apparent distribution and elimination phases (α, β), the corresponding half-life for each rate constant, the maximum plasma concentration (C_{max}), the time needed to reach C_{max} (t_{max}), the total mean residence time (*MRT*), and the apparent oral clearance (Cl_{po}). The Cl_{po} was calculated by dividing the dose by *AUC*. All calculated values are given as means $\pm$ SD, except for $t_{½\beta}$ values, which are expressed as harmonic means $\pm$ 'pseudo SD'. The statistical analysis of data was performed using the one-way analysis of variance (ANOVA) followed by multiple comparison between data for chickens, turkeys and geese. A difference at $P < 0.05$ was considered significant.

The mean norfloxcin plasma concentration–time profiles in chickens, turkeys and geese are presented in Fig. 1. As the curves show the plasma concentrations of norfloxacin decreased in a biexponential manner in each species. A good fit of the observed data to a two-compartment open model was obtained.

In chickens, norfloxacin appeared in plasma within a short period of time with a mean maximum concentration (C_{max}) of 1.46 ± 0.18 μg/mL reached at 1.99 ± 0.17 h after oral dosing of the drug. Plasma concentrations exceeding 0.25 g/mL, the MIC_{90} against the major poultry pathogenic gram-negative bacteria (*E. coli, Salmonella spp., Pasteurella spp.*) and mycoplasmas (Hannan *et al.*, 1989; Prescott & Baggot, 1993) were maintained between 0.5–10 h after administration of the drug. The elimination half-life was 11.1 h indicating slow final disappearance of the norfloxacin from blood. Due to the relatively high peak concentration and slow elimination, the calculated value for area under the concentration–time curve was quite large (*AUC*: 10.41 ± 1.22 h·μg/mL) with an oral clearance of 0.97 ± 0.13 L/h·kg. Our findings obtained in chickens partly differ from those reported by Anadón *et al.* (1992) who found much faster absorption ($t_{½a}$: 0.05 h, t_{max}: 0.22 h) and a higher peak concentration (C_{max}: 2.89 μg/mL) of norfloxacin after a single oral dose of 8 mg/kg in chickens. The reason for these differences is not clear and several factors might be involved, e.g. in the referred study the sodium salt of norfloxacin was administered and we used a commercial product containing the norfloxacin base.

As regards the appearance profile (C_{max}, t_{max}) of norfloxacin in blood, our results are, however in good agreement with those obtained in dogs (Brown *et al.*, 1990), monkeys (Gilfillan *et al.*, 1984) and humans (Swanson *et al.*, 1983) or reported for enrofloxacin, a related fluoroquinolone compound, in chickens (Anadón *et al.*, 1995).

In turkeys, the measured plasma concentrations were considerably lower than in chickens, however, the rate of appearance of the drug in blood and the time to reach mean maximum

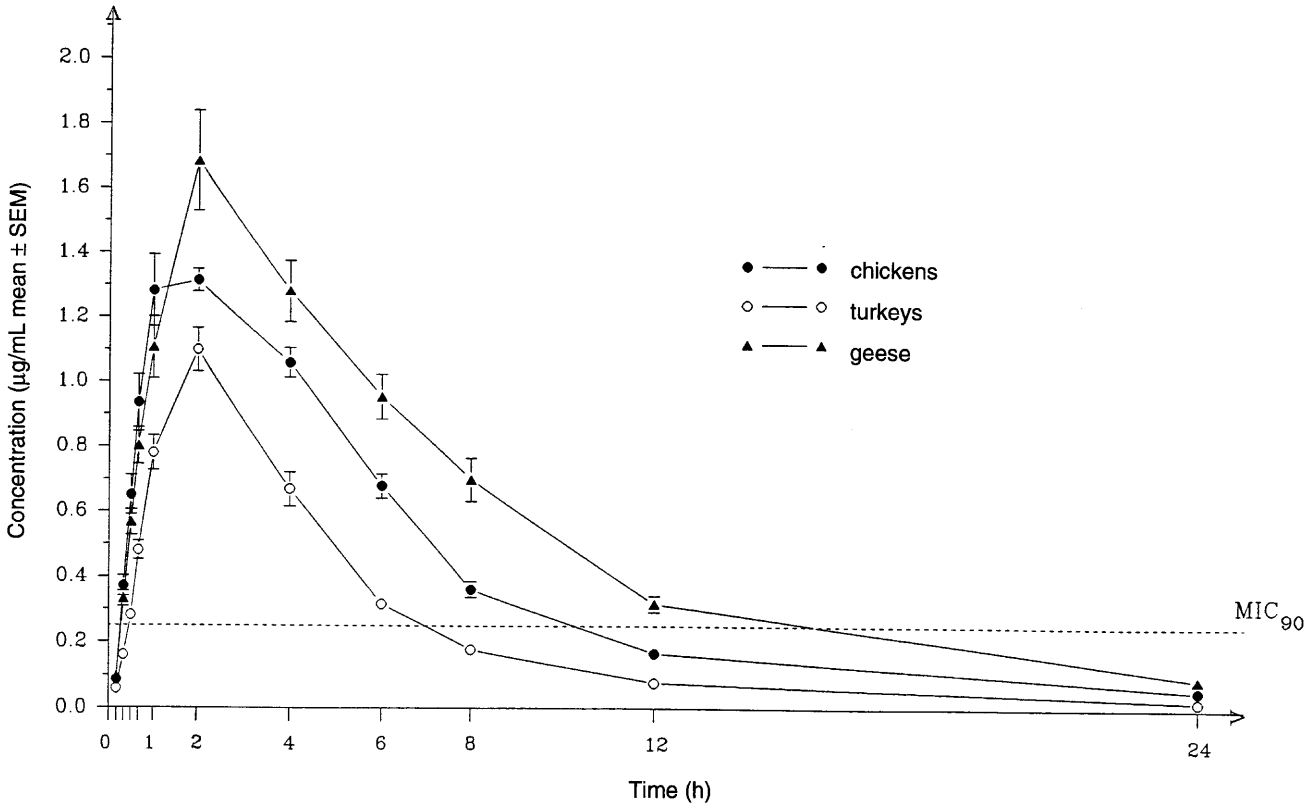

Fig. 1. Mean plasma concentrations of norfloxacin in chickens, turkeys and geese. Data are expressed as mean $\pm$ SEM for 6 animals in each species.

Table 1. Mean pharmacokinetic parameters (mean ± SD) of norfloxacin in chickens, turkeys and geese after a single oral administration of 10 mg/kg

Parameter	Chicken $n = 6$	Turkey $n = 6$	Goose $n = 6$
α (h^{-1})	0.40 ± 0.05^a	0.49 ± 0.02^b	0.27 ± 0.03^c
β (h^{-1})	0.06 ± 0.01^a	0.08 ± 0.02^a	0.07 ± 0.01^a
k_a (h^{-1})	0.79 ± 0.23^a	0.75 ± 0.05^a	0.77 ± 0.15^a
$t_{½a}$ (h)	0.92 ± 0.21^a	0.93 ± 0.06^a	0.93 ± 0.18^a
$t_{½\alpha}$ (h)	1.75 ± 0.22^a	1.43 ± 0.07^a	2.58 ± 0.27^b
$t_{½\beta}$ (h)	11.11 ± 2.28^a	9.07 ± 1.00^a	10.65 ± 2.41^a
k_e (h^{-1})	0.28 ± 0.04^a	0.37 ± 0.05^b	0.19 ± 0.02^c
C_{max} (μg/mL)	1.46 ± 0.18^a	0.95 ± 0.15^b	1.58 ± 0.30^a
t_{max} (h)	1.99 ± 0.17^a	1.94 ± 0.08^a	2.39 ± 0.18^b
AUC (h·μg/mL)	10.41 ± 1.22^a	5.97 ± 0.98^b	14.78 ± 3.03^c
MRT (h)	8.82 ± 1.02^a	6.64 ± 1.08^b	9.96 ± 1.16^a
Cl_{po} (L/h·kg)	0.97 ± 0.13^a	1.71 ± 0.29^b	0.70 ± 0.14^a

α, β = hybrid rate constants for apparent distribution and elimination phases; k_a = first-order rate constant for appearance of the drug in blood; k_e = rate constant for disappearance from the central compartment; $t_{½a}$ = absorption half-life; $t½\alpha$, $t½\beta$ = half-lives at α and β phases; C_{max} = maximum plasma concentration calculated; t_{max} = time to reach C_{max}; *AUC* = area under the concentration–time curve; *MRT* = mean residence time; Cl_{po} = apparent oral clearance; ^{a,b,c} = means with no common superscript within each row are significantly different (Bonferroni P value < 0.05).

plasma concentration (t_{max}: 1.94 ± 0.08 h) were very close to those obtained in chickens. Plasma concentrations above 0.25 μg/mL were attained for 6 h after dosing. The calculated C_{max}, *AUC* and *MRT* were 0.95 ± 0.15 μg/mL, 5.97 ± 0.98 h·μg/mL and 6.64 ± 1.08 h, respectively. These values were significantly smaller than those obtained in chickens and geese. Without i.v. data it can not be stated with certainty, but considering the relevant kinetic parameters it can be assumed that a reduced bioavailability due to a lower extent of absorption and/or pronounced first pass metabolism or a quicker elimination might contribute equally to the different plasma concentration–time profiles in animal species investigated.

In turkeys, a single oral dose of 40 mg/kg norfloxacin nicotinate was reported to provide a maximum plasma concentration of 0.88 μg/mL at 0.7 h (Gulkarov & Ziv, 1994) also suggesting relatively low plasma concentrations of the drug in this animal species.

In geese, norfloxacin appeared in blood in somewhat higher concentrations than in chickens, and mean C_{max} (1.58 ± 0.3 μg/mL) was significantly greater than in turkeys. The plasma concentrations of norfloxacin remained above the MIC_{90} of norfloxacin for avian pathogenic bacteria for more than 12 h. The mean peak concentration was achieved significantly later (t_{max}: 2.39 ± 0.18 h) than in chickens and turkeys. Additionally, the *AUC* (14.78 ± 3.03 h·μg/mL) was also significantly different from those obtained in chickens and turkeys. For geese, no reported data about the pharmacokinetics of norfloxacin were available. Considering the rate constant for disappearance it seems probable that the elimination of norfloxacin in geese is slower than in chickens and turkeys, however, this should be corroborated by further studies.

The fluoroquinolones exert their bactericidal effect against gram-negative bacteria in a concentration-dependent manner and they show a moderate post-antibiotic effect (Craig & Elbert, 1991; Keck & Borne, 1995), thus the administration of the total daily dose of enrofloxacin to chickens over a 2–4 h period in water (pulse dosing) has been recommended (Stegemann, 1995). The results obtained in the present studies indicate that norfloxacin has useful pharmacokinetic properties for oral use in chickens, turkeys and geese. It provides therapeutic plasma concentrations in each species, the extent and duration of which have been found to be greatest in geese and lowest in turkeys. The high C_{max}/MIC ratio and the long elimination half-life of the drug obtained in geese and chickens would allow a once-per-day pulse-dose schedule for norfloxacin administration (to be developed) in the drinking water for these species. In turkeys, however, the applied dose ensured plasma concentrations above the MIC_{90} of norfloxacin for relevant pathogens only for 6–8 h. In establishing the dosage regimen of fluoroquinolones it should also be considered that mutational resistance may develop with a great frequency at sub MIC concentrations (Semjén & Wright, 1991). Consequently, the once-per-day application of norfloxacin at a dose of 10 mg/kg to turkeys does not seem to be sufficient. Recently, a higher dose of sarafloxacin has been proposed for turkeys than for chickens but the method of application (pulse or continuous dosing) was not mentioned (Brown, 1996). Similarly the dose of norfloxacin for turkeys should probably also be increased. As the product investigated in the present study is easily soluble in water and the solution is stable for 24 h both the pulsing and continuous administrations are conceivable.

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