Integration of Pharmacokinetic and Pharmacodynamic Indices of Marbofloxacin in Turkeys

Running title: PK-PD of marbofloxacin in turkeys

Aneliya Milanova Haritova, Nikolina Velizarova Rusenova, Parvan Rusenov Parvanov, Lubomir Dimitrov Lashev, Johanna Fink-Gremmels

1. Department of Veterinary Pharmacology, Pharmacy and Toxicology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

2. Department of Pharmacology, Physiology and Chemistry, Faculty of Veterinary Medicine, Trakia University, Bulgaria

3. Department of Microbiology, Infectious and Parasitic Diseases, Section of Microbiology, Faculty of Veterinary Medicine, Trakia University, Bulgaria

* Utrecht University

Faculty of Veterinary Medicine,
Department of Pharmacology, Pharmacy and Toxicology,
Yalelaan 16, De Uithof, P.O.Box 80152,
3508 TD Utrecht,
The Netherlands

Phone + 3130 2535453
Fax + 3130 2534125
E-mail: J.Fink@vet.uu.nl
Abstract

Fluoroquinolones are extensively used in the treatment of systemic bacterial infections in poultry, among others for systemic *E. coli* bacillosis that is a common disease in turkey flocks. Marbofloxacin has been licensed for use in various mammalian species, but as yet not for turkeys, although its kinetic properties distinguish it from other fluoroquinolones. For example, the longer half-life of marbofloxacin in many animal species has been appreciated in veterinary praxis.

It is generally accepted that for fluoroquinolones the optimal dose should be estimated on the basis of pharmacokinetic and pharmacodynamic characteristics of the drug under consideration. Knowledge of these specific data in the target animal species allows establishing an integrated PK-PD model that is of high predictive value. In the present study, the antibacterial efficacy (PD indices) against a field isolate of *Escherichia coli* O78/K80 was investigated *ex vivo* following oral and intravenous administration of marbofloxacin to turkeys (breed BUT 9, 6 animals per group) at dose of 2 mg/kg b.w. At the same time, the plasma concentrations of marbofloxacin were measured at different time intervals by a standardized HPLC method, allowing the calculation of the most relevant kinetic parameters (PK parameters).

The *in vitro* Inhibitory Activity$_{serum}$ of marbofloxacin against the selected *E. coli* O78/K80 strain was 0.5 μg/ml in blood serum of turkeys, and the value of C$_{max}$/Inhibitory Activity$_{serum}$ ratio was 1.34. The lowest Concentration$_{24h}$/InhibitoryActivity$_{serum}$ required for bacterial elimination was lower than AUC/InhibitoryActivity$_{serum}$. These first results suggested that the recommended dose of 2 mg/kg b.w. marbofloxacin is sufficient to achieve a therapeutic effect in diseased animals. However, considering the risk of resistance induction, the applied
dose should be equal to AUC/MICs>125, the generally recommended dose for all fluoroquinolones. According the presented PK-PD results a dose 3.0-12.0 mg/kg b.w. per day would be needed to meet this criterion. In conclusion, the results of the present study provide the rationale for an optimal dose regimen for marbofloxacin in turkeys and hence should form the basis for dose selection in forthcoming clinical trials.

**Key words:** marbofloxacin; turkey; PK-PD model
Introduction

Marbofloxacin is a synthetic fluoroquinolone, developed for veterinary use only (47). Belonging to the third generation of quinolones it has a broad spectrum of activity (58), and a bactericidal concentration-dependent killing is observed against many Gram-negative bacteria (12, 49, 51, 52). The pharmacokinetic properties of marbofloxacin have been studied in several mammalian species, and some advantages over other fluoroquinolones, such as a longer elimination half-life were described (2, 43, 47, 48). In practice, this would enable a single treatment per 24 h, with serum concentrations remaining above MIC for more than 12 hours. Comparable kinetic data are lacking, however, for turkeys as yet.

Fluoroquinolones are used in poultry predominantly with the aim to control systemic colibacillosis (13, 18, 31, 56). The efficacy of this class of drugs against colibacillosis has been tested under field conditions, but results are based solely on monitoring of the clinical outcome (10, 11, 13, 14, 24, 50). The weak point of this approach is that in field trials spontaneous clinical recovery often masks differences in bacteriological efficacy of antibacterial drugs (Pollyanna effect), resulting in the use of sub-optimal dose regimens, and hence increasing the risk of resistance induction. Particularly in poultry, sub-optimal antibacterial therapy comprises a risk for human health, as resistant zoonotic bacteria, like Salmonella spp., Campylobacter spp. and VTEC (verotoxin-producing E. coli) may reach the consumer (17, 29, 30, 44). Thus therapeutic regimes need to be critically reviewed, correlating bacterial cure rates with the risk for selection and spread of resistant pathogens.

The clinical success of a given therapy depends on the relationship between the pharmacokinetic (PK) and pharmacodynamic (PD) properties of a drug (38, 57). The integration of PK (bioavailability and clearance) and PD (MIC) indices allows to
predict efficacy and potency of a drug in the early phase of drug development and supports post-marketing surveillance (52, 54). Hence, PK-PD models serve to select the optimal drug dosage and the more specific selection of an appropriate antimicrobial within the given class of antibiotics (9, 34, 37, 53). Increasing evidence suggests that the main PK-PD surrogates for fluoroquinolones correlating with clinical cure and bacterial eradication are the AUC/MIC and $C_{\text{max}}$/MIC ratios (9, 46). Hence this approach determines *ex vivo* PK-PD indices, which subsequently allow a more targeted design in confirmatory *in vivo* studies (1, 2, 3). PK-PD experiments with marbofloxacin were previously conducted in calves, cows, goats and dogs (2, 43, 48; 55). Moreover, pharmacokinetic data for marbofloxacin have been estimated for chickens and Eurasian buzzards (6, 19). However, there are no reports about PK and PK-PD indices in turkeys, and the advantages or possible disadvantages of marbofloxacin in comparison to other fluoroquinolones were not evaluated yet.

Hence, the aim of the present study was to estimate the PK-PD surrogates required for bacteriostasis, bactericidal activity and bacterial elimination as described by Aliabadi et al. (1) and Toutain et al. (54) for marbofloxacin in turkeys after oral administration, as these data provide a basis for the suggestion of optimal therapeutic dose regimes.

### Material and Methods

#### Drugs

Marbofloxacin (Marbocyl 10 % injectable solution, Vetoquinol, Batch No 130300/1205 PdA1, V1205) was used for i.v. treatment. The same sterile formulation was diluted with sterile pyrogen-free water to 1% w/v and than used for oral administration.
Animals

Six clinically healthy turkeys (breed BUT 9), 8 months old were included in the experiments. Three birds were male and three were female, with a body weight of 9.9-10.12 kg and 6.08-6.96 kg, respectively. The animals were obtained from an experimental poultry farm in Stara Zagora, Institute of Animal Husbandry.

Animals were housed under identical conditions (at 20°C), according to the requirements for this species. Standard commercial feed (without antibiotics and coccidiostats) and water were supplied ad libitum.

Study design

A two-way crossover design was used, with a washout period of 15 days between individual treatments. The i.v. administration was given in the V. brachialis, the oral administration by installation of the marbofloxacin solution into the crop via a plastic tube, after 12 hours food deprivation. Blood samples were collected from the V. brachialis. After i.v. administration, blood samples were collected from the contralateral vein.

Marbofloxacin was administered i.v. and orally at a dose rate of 2 mg/kg b.w., according to the manufacturer’s instructions for other animal species. Blood samples were collected prior to each treatment and at 0.083, 0.25, 0.5, 1, 2, 3, 6, 9, 12, 24, 36 and 48 h after the i.v. administration. Blood samples after oral administration were collected prior to each treatment and at 0.25, 0.5, 0.75 (1 ml) and at 1, 1.5, 2, 3, 6, 9, 12, 24, 36 and 48 h (1.5 ml) after dosing. Samples were collected without anticoagulant and kept at room temperature for 2 h in the dark. Serum was collected after centrifugation at 1800×g for 15 min and stored at -25°C prior to analyses.

Determination of MIC and MBC values
(a) Bacterial isolates

The MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) values were determined with an *Escherichia coli* O78/K80 strain, isolated from turkeys, that was obtained from the National Scientific and Diagnostic Institute of Veterinary Medicine, Sofia, Bulgaria. The used *E. coli* strain was stored on beads at -20°C prior to use. *E. coli* was grown on tryptone Soya blood agar (TSA; Becton Dickinson and Co, Difco Laboratories, Le Pont de Claix, France; Ref. No 236950). Colonies from overnight growth were directly suspended in Mueller-Hinton broth (MHB; Becton Dickinson and Co, Difco Laboratories, Le Pont de Claix, France; Ref. No 275730) to obtain a turbidity comparable to that of the 0.5 McFarland turbidity standard. Cultures were diluted 1:100 with broth to obtain a dilution of $10^6$ CFU/ml.

(b) MIC determination and activity in serum

Marbofloxacin solutions at twice the required final concentration of 128 μg/ml were added either to MHB (according to NCCLS, 2000, 41) or to blood serum obtained from control animals. Serial dilutions from this solution were prepared in broth and in serum with concentration ranging between 64 μg/ml and 0.0156 μg/ml and were inoculated with approximately $5 \times 10^5$ CFU/ml *E. coli* O78/K80. Tubes were incubated at 35°C for 18 h and then shaken to mix the contents.

An aliquot of 100 μl from each tube was subcultured on TSA, and the plates were incubated at 35°C overnight and the colonies counted. The limit of detection was 10 CFU/ml. MIC and Inhibitory Activity$_{serum}$ were defined as the lowest concentration at which bacterial growth remained below the level of the original inoculum. MBC
and Bactericidal Activity$_{serum}$ were defined as the concentration at which a 99.9% reduction in the bacterial counts was achieved.

**Antimicrobial activity in the serum of animals treated with marbofloxacin**

Eight to ten colonies from overnight growth of *E. coli* in TSA (as mentioned above) were used to inoculate 9 ml of MHB, and the colonies were allowed to grow overnight at 35°C.

To 0.5 ml serum from treated animals 5 µl of the stationary phase of bacterial cultures was added, to give a final concentration of approximately $3 \times 10^7$ CFU/ml.

To determine the number of CFU, serial dilutions were prepared with sterile saline (ranging from $10^{-2}$ to $10^{-6}$ CFU/ml) and incubated for 3, 6, and 24 h. Thereafter an aliquot of 20 µl was plotted on TSA plates and CFUs were counted after 16 h incubations. The limit of detection was 10 CFU/ml.

**Determination of marbofloxacin serum concentrations**

**HPLC method**

The serum concentrations of marbofloxacin were determined by HPLC according to the method of analysis as described by Garcia et al. (20). Standard solutions were prepared in serum obtained from untreated turkeys at concentrations of 2.5, 1.0, 0.5, 0.2, 0.1, 0.05, 0.025, 0.02 (LOQ) and 0.01 (LOD) µg marbofloxacin per ml. The value of $r$ for the standard curve was 0.998 and the linearity was confirmed by the test for lack of fit (P=0.653). The intra-assay and the inter-assay coefficients of variation (CV) for marbofloxacin were calculated to be 9.18 and 5.87, respectively.

**Microbiological assay**
Parallel to the HPLC determinations, the concentrations of marbofloxacin were measured by agar-gel diffusion method using *Escherichia coli* ATCC 25922 as test microorganism. The nutrient medium was meat-peptone agar (National Research Institute of Infectious and Parasitic Diseases, Sofia, Bulgaria). Standard solutions were prepared in serum obtained from untreated animals. The value of $r$ for the standard curve was 0.993 and the linearity was confirmed by the test for lack of fit ($P=0.749$). The intra-assay CV was 9.09 and the inter-assay CV was 10.60. The limit of quantification in serum samples was 0.04 µg/ml.

**Pharmacokinetic analysis**

Pharmacokinetic analysis of the data was performed using non-compartmental analysis based on statistical moments theory (21) (WinNonlin 4.0.1., Pharsight Corporation, 800 West El Camino Real, Mountain View, CA, USA). The weighting scheme $1/y^2$ was used. The area under the serum concentration-time curve (AUC) was calculated by the trapezoid rule with extrapolation to infinity. The absolute bioavailability was calculated using the following equation:

\[
(1) \quad F_{\text{abs}}\% = \left(\frac{\text{AUC}_{\text{po}}}{\text{AUC}_{\text{iv}}}\right) \times 100.
\]

**Pharmacodynamic analysis**

AUC/MIC and AUC/MBC values were obtained on the basis of the area under the concentration-time curve over 24 hrs divided by the MIC and MBC, respectively, which were determined in broth (40). Concentration$_{24\text{h}}$/InhibitoryActivity$_{\text{serum}}$ and Concentration$_{24\text{h}}$/BactericidalActivity$_{\text{serum}}$ ratios were also determined. In these indices Concentration$_{24\text{h}}$ (estimated by multiplying the measured serum concentration by incubation period of 24 hrs) was divided by InhibitoryActivity$_{\text{serum}}$ and...
BactericidalActivity\textsubscript{serum}, respectively, as determined in serum. \text{C\text{\textsubscript{max}}/InhibitoryActivity\textsubscript{serum} and C\text{\textsubscript{max}}/BactericidalActivity\textsubscript{serum} values were estimated by using InhibitoryActivity\textsubscript{serum} and BactericidalActivity\textsubscript{serum} values that were determined in serum (1,2,3) and were used for PK-PD integration in this study. The log10 difference between the initial bacterial count (in number of CFU per millilitre) and the bacterial count after 24 h of incubation was also determined for turkey serum. To calculate the Concentration\textsubscript{24h}/InhibitoryActivity\textsubscript{serum} ratio in the effect compartment required for bacteriostic and bactericidal activities, and for the total elimination of bacteria, the sigmoid inhibitory \text{E\textsubscript{max}} model was used. Antibiotic response (expressed in terms of reduction of the initial bacterial count) is regressed against the surrogate marker (Concentration\textsubscript{24h}/InhibitoryActivity\textsubscript{serum}) using the Hill equation:

\begin{equation}
E = E\text{\textsubscript{max}} - \left\{ \frac{(E\text{\textsubscript{max}} - E_0) \times C_e^N}{(E_{I50}^N + C_e^N)} \right\},
\end{equation}

where \text{E} is the antibacterial effect measured as the change in the bacterial counts (in log10 CFU per millilitre) in the serum sample after 24 h of incubation compared to the initial log10 CFU per millilitre; \text{E\textsubscript{max}} is the log10 difference in bacterial counts between 0 and 24 h in the control sample; \text{E}_0 is the log10 difference in bacterial counts in the test sample containing marbofloxacin after 24 h of incubation when the limit of detection of 10 CFU/ml is reached; \text{E}_{I50} is the Concentration\textsubscript{24h}/InhibitoryActivity\textsubscript{serum} producing 50% of the maximal antibacterial effect; \text{C}_e is the Concentration\textsubscript{24h}/InhibitoryActivity\textsubscript{serum} in the effect compartment (serum); and \text{N} is the Hill coefficient, which describes the steepness of the (Concentration\textsubscript{24h}/InhibitoryActivity\textsubscript{serum})-effect curve. In these investigations, \text{E\textsubscript{max}} represents the baseline bacterial count and \text{E}_0 is the maximal effect because the drug inhibits bacterial growth (1, 2, 5). Hence the antibacterial response is the dependent
variable representing the reduction of the initial bacterial count. The independent
variable is the surrogate Concentration\textsubscript{24h}/InhibitoryActivity\textsubscript{serum} value. These PD
indices were calculated on the basis of all samples by using the WinNonlin nonlinear
regression program.

PK-PD analysis

By using \textit{in vitro} MIC data and \textit{in vivo} PK parameters, the surrogate markers of
antimicrobial efficacy, \(C_{\text{max}}/\text{MIC}\), AUC/MIC, and \(T_{>\text{MIC}}\) were determined for serum
after both, i.v. and oral administration of marbofloxacin. Because it is not possible to
obtain large volumes of blood from turkeys, the PK-PD simulations were done on the
basis of all values obtained from treated animals.

Antibacterial efficacy was quantified from the sigmoid \(E_{\text{max}}\) equation (Equation
2) by determining Concentration\textsubscript{24h}/InhibitoryActivity\textsubscript{serum} required for a bacteriostatic
effect (no change in bacterial count after 24 h of incubation), a 50% reduction in the
bacterial count, a bactericidal effect (a 99.9% decrease in the bacterial count), and for
the bacterial elimination (the lowest Concentration\textsubscript{24h}/InhibitoryActivity\textsubscript{serum} that
produced a reduction in bacterial counts to 10 CFU/ml) in serum (1).

Statistical analyses

The pharmacokinetic parameters of marbofloxacin were presented as mean ±
standard deviation. They were computed with the Statistica 6.1 computer program
(Statistica for Windows, StatSoft, Inc., USA, 1984-2002). A statistical analysis of the
data obtained from the microbiological assays and from HPLC analysis was carried
out using the Wilcoxon test. A value of \(P<0.05\) was considered significant. The same
program was used for statistical analysis of the standard curves.
Results

MIC and MBC values for marbofloxacin. The MIC (0.125 µg/ml) and MBC (0.5 µg/ml) values were 4-fold lower than the values for the InhibitoryActivity_{serum} (0.5 µg/ml) and BactericidalActivity_{serum} (2.0 µg/ml).

Intravenous administration of marbofloxacin.

Data show the results of the HPLC determination, as there was no significant difference between HPLC results (Fig. 1) and the results from the microbiological assay (data not shown). A summary of the kinetic parameters is given in Table 1. The PK-PD integration index AUC/InhibitoryActivity_{serum} resulting from in vivo kinetics and in vitro InhibitoryActivity_{serum} values for marbofloxacin was 23.58 (versus AUC/MIC of 94.32). These results indicate that the concentrations in serum exceed InhibitoryActivity_{serum} values (0.5 µg/ml) over a period of 9 hours.

Oral administration of marbofloxacin.

Peak serum concentrations were found between 3 and 6 h after drug administration, and the estimated MAT (mean absorption time) was 4.97±2.59 h. After 48 h the residual concentrations were close to the limit of quantification (Fig. 1 and Table 1). The AUC/InhibitoryActivity_{serum}, was approximately 18 (18.4±6.4) and C_{max}/InhibitoryActivity_{serum} was 1.34±0.58. The values of AUC/MIC (73.69±25.54) and C_{max}/MIC (5.35±2.31), were nearly 4 times higher. The value of T_{>MIC} was 10.9 h.

Antibacterial activity in serum of animals treated orally with marbofloxacin. The activity of marbofloxacin against E. coli in serum of treated animals was determined
and prominent inhibitory effect was observed for samples taken between 3 and 12 h, whereas at 24 and 36 h no significant inhibition of bacterial could be measured. The antibacterial time-dependent-killing curves are presented in Fig. 2. This figure presents with the control values (taken at 0 hours) the log-normal growth curve of the E.coli test strain in serum from untreated turkeys, that have to be compared with the bacterial growth curves in serum samples taken at the indicated time intervals after treatment.

Calculation of Concentration$_{24h}$/InhibitoryActivity$_{serum}$ required for a bacteriostatic or bactericidal activity, and for total elimination of bacteria. Graphs depicting the bacterial counts and Concentration$_{24h}$/InhibitoryActivity$_{serum}$ relationships for serum for 24 h are presented in Fig. 3. The lowest Concentration$_{24h}$/InhibitoryActivity$_{serum}$ required for bacterial elimination was lower than AUC/InhibitoryActivity$_{serum}$. The steep slope of the Concentration$_{24h}$/InhibitoryActivity$_{serum}$-versus-bacterial count relationship explains the relatively similar values calculated for Concentration$_{24h}$/InhibitoryActivity$_{serum}$ ratios that produced bacteriostatic or bactericidal activity (Table 2).

Discussion

Data on pharmacokinetics of marbofloxacin in poultry is limited, and specific pharmacokinetic data for turkeys are lacking. Hence, turkeys were treated with marbofloxacin at the recommended dose of 2 mg/kg b.w., either by i.v. or by oral route. The serum concentrations were measured by two independent methods, a standardized HPLC method allowing the quantification of parent marbofloxacin, and a bioassay measuring antimicrobial activity in serum samples from treated animals.
This microbiological assay would detect also any biologically active metabolites of marbofloxacin. Data show that the results obtained with both assays are very comparably. This good correlation indicates that the metabolism of marbofloxacin in turkeys is limited and suggests that any formed metabolite is less or not microbiologically active, which is in agreement with previous studies (6).

Following i.v. injection, the value of $t_{1/2\beta}$ of marbofloxacin was longer in turkeys than in broilers (5.26 h) and buzzards (4.11 h) (6, 19). In comparison with other fluoroquinolones (enrofloxacin, danofloxacin, fleroxacin, ofloxacin), marbofloxacin has a lower volume of distribution and a longer elimination half-life (2, 7, 8, 32, 35). The calculated mean absorption time (MAT) suggests a rather slow absorption of the drug after oral administration, but the calculated bioavailability indicates a high rate of absorption (F=84.4%). In chickens, marbofloxacin was absorbed to a lower extent (F=56.8%), but $C_{\text{max}}$ was detected earlier (6). In comparison to danofloxacin (F=78.4%) and enrofloxacin (F=69.85%), marbofloxacin has a higher oral bioavailability (25, 26). The oxadiazine cycle in the molecule of marbofloxacin, which makes it different from other fluoroquinolones, seems to determine the higher oral bioavailability and the increased elimination half-life. In other studies with marbofloxacin in various animal species it was concluded that the pharmacokinetic properties of marbofloxacin seems to be advantageous as compared to over other fluoroquinolones (2, 7, 8, 32, 35).

The most frequently used pharmacodynamic index for measuring the activity of an antimicrobial in vitro is the estimation of the MIC, and this value is used to predict the antimicrobial efficacy and potency of a drug. Although, MIC and InhibitoryActivity$_{\text{serum}}$ values are comparable to published data for MIC$_{90}$ values of most pathogenic $E.\ coli$ strains (6, 49), it should be reiterated that growth curves (and
MIC values) measured in broth only, are less representative than that determined in serum or even in vivo findings. Our finding that in the presence of serum, the InhibitoryActivity_{serum} was reduced (resulting in values that exceeded the MIC in standard broth by approximately a factor of 4) coincides with previously reported data on the decreased antimicrobial activity of most fluoroquinolones in the presence of serum (2-4 fold higher MIC values; 4, 5, 25, 28, 57). Protein binding explains the lower inhibitory activity of some fluoroquinolones in serum (57), but compared to other fluoroquinolones marbofloxacin has a rather low rate of plasma protein binding; hence other factors may contribute as well to the observed differences (39).

PK-PD indices in the current study were used according to the standardized terminology and other terms were defined when these indices differ from the generally accepted definitions (40). Clinical investigations in human medicine and animal studies have shown that AUC/MIC and C_{max}/MIC correlate strongly with the clinical response to fluoroquinolones with better predictive value of the first ratio (42, 46). The calculated values of C_{max}/InhibitoryActivity_{serum} for marbofloxacin (1.34 – 1.58) were lower the comparable values for danofloxacin mesylate (4.06) for the investigated strain E.coli O78/K80 (26), reflecting the lower potency of marbofloxacin. For danofloxacin the C_{max}/InhibitoryActivity_{serum} ratio obtained with the recommended therapeutic dose (6 mg/kg b.w., orally) results in a 99% reduction in bacterial counts (15, 26, 45). The results presented here for marbofloxacin and previously published data for enrofloxacin in turkeys (C_{max}/MIC - 1.7) suggest a higher survival rate of pathogens, hence indicating a risk for development of antimicrobial resistance against fluoroquinolones in turkeys (16, 22, 25).

The steep slope of Concentration_{24h}/InhibitoryActivity_{serum} versus bacterial count curves with a high Hill coefficient and in vitro investigations demonstrate that
marbofloxacin, like danofloxacin, exerts a concentration-dependent killing against different strains of *E. coli* (48, 54). However, the antibacterial activity of marbofloxacin against *E. coli* O78/K80 in serum (determined as log10 CFU/ml difference in bacterial count in the test sample containing marbofloxacin) appeared to be slower during the first six hours of incubation, as compared to danofloxacin (26). Bacterial elimination could be achieved with danofloxacin at lower Concentration24h/InhibitoryActivityserum ratios in comparison to marbofloxacin (26). Marbofloxacin, however, possess some preferable pharmacokinetic properties in comparison to other fluoroquinolones such as low serum protein binding and ClB, which should compensate for the lower activity against *E. coli* O78/K80 (25, 26).

Applying the integrated PK and PD approach and the estimated surrogates and using the equation proposed by Toutain et al. (54) (Dose=(AUC/MIC×ClB×MIC)/F) the calculated dose for marbofloxacin equals 1.2 mg/kg b.w. per 24 h. Considering also the AUC/InhibitoryActivityserum value (18.42 h) achieved with the recommended dose for marbofloxacin of 2 mg/kg, it can be assumed that this fluoroquinolone could be an appropriate choice to achieve clinical cure of *E. coli* infections. A remaining limiting variable is the varying intrinsic sensitivity of field isolates of *E. coli* against marbofloxin, as in our approach the PD data (i.e. MIC and MBC values) were determined only in one individual strain. McKellar et al. (36) suggested incorporating MIC90 and MIC values from one strain in the PK-PD calculation as indicative for the variability of *E. coli* isolates. A prerequisite, however, is the availability of representative data in this case from different *E. coli* strains isolated from turkeys. Other factors which also could influence the outcome of treatment such as immunity status of birds, physiological changes during infection, tissue distribution of drug are not considered in the PK-PD modelling.
The therapeutic use of fluoroquinolones in poultry is only assessed in terms of good clinical efficacy, but needs to consider the risk of the induction of antimicrobial resistance, as zoonotic pathogens like *Salmonella spp.* and *Campylobacter spp.* are prevalent in poultry flocks and can be transmitted via meats to consumers (18). Gunderson et al. (23) and Hyatt et al. (27) recommended that for the treatment of Gram-negative infections higher AUC/MIC ratios, along with high values for C<sub>max</sub>/MIC and T<sub>T>MIC</sub> surrogates, should be used to reduce the risk of resistance induction. These authors recommend a breakpoint of 125 (AUC/MIC > 125) to reduce the risk of emergence of resistance (23, 46, 57).

Following the paradigm that the AUC/MIC ratio should exceed a value of 125, reaching under optimal conditions even a ratio of 400-500 (27), the data presented would suggest optimal doses of 3.0 up to 12.0 mg/kg b.w. per day for marbofloxacin if MIC is 0.125 µg/ml. This suggestion is in line with the proposed higher dose regimens for danofloxacin, enrofloxacin, sarafloxacin and norfloxacin in turkeys (25, 26, 33). As was already mentioned above, the applied approach has limitations since the activity of marbofloxacin was not determined here in challenge experiments, and PK-PD indices serve as surrogate markers for efficacy. Therefore, clinical trials should validate this dose in diseased turkey flocks under practical conditions, assessing not only bacteriological cure rates, but also monitor the emerge of antibacterial resistance.

Acknowledgements

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References


**Table 1. Pharmacokinetic parameters (non-compartmental analysis) of marbofloxacin in turkeys after i.v. and oral administration, respectively, of 2 mg marbofloxacin/kg b.w.** Data represent mean±SD of 6 individual animals.

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<tr>
<th>Pharmacokinetic parameters</th>
<th>Units</th>
<th>HPLC analysis</th>
<th>Microbiological assay</th>
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</thead>
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<tr>
<td><strong>Non-compartmental analysis – intravenous application</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>h</td>
<td>7.37 ±1.66</td>
<td>9.01 ±3.14</td>
</tr>
<tr>
<td>$\text{Cl}_B$</td>
<td>ml.h$^{-1}$.kg$^{-1}$</td>
<td>158.4 ±27.5</td>
<td>116.6 ±45.22</td>
</tr>
<tr>
<td>$V_{d_{\text{area}}}$</td>
<td>l.kg$^{-1}$</td>
<td>1.66 ±0.34</td>
<td>1.75 ±0.25</td>
</tr>
<tr>
<td>$V_{ss}$</td>
<td>l.kg$^{-1}$</td>
<td>1.41 ±0.25</td>
<td>1.54 ±0.19</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>9.04 ±1.71</td>
<td>11.29 ±3.67</td>
</tr>
<tr>
<td>$\text{AUC}_{0-24h}$</td>
<td>µg.h.ml$^{-1}$</td>
<td>11.79 ±1.97</td>
<td>13.41 ±2.64</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty}$</td>
<td>µg.h.ml$^{-1}$</td>
<td>12.94 ±2.21</td>
<td>16.71 ±5.36$^*$</td>
</tr>
</tbody>
</table>

| **Non-compartmental analysis - oral application** | | | |
| MRT | h | 14.01 ±3.38 | 11.69 ±2.54 |
| $\text{AUC}_{0-24h}$ | µg.h.ml$^{-1}$ | 9.21 ±3.19 | 10.07 ±3.59 |
| $\text{AUC}_{0-\infty}$ | µg.h.ml$^{-1}$ | 10.89 ±3.21 | 10.86 ±3.45 |
| $t_{1/2\beta}$ | h | 7.73 ±1.00 | 6.23 ±1.63 |
| $C_{\text{max}}$ | µg/ml | 0.67 ±0.29 | 0.80 ±0.32 |
| $T_{\text{max}}$ | h | 6.0 ±3.29 | 6.50 ±3.51 |
| $F_{\text{abs}}$ | % | 84.37 ±21.26 | 70.67 ±30.66 |

$t_{1/2\beta}$ - terminal elimination half-life; $\text{AUC}_{0-\infty}$ - area under the serum concentration-time curves from 0 h to $\infty$; $\text{AUC}_{0-24h}$ - area under the serum concentration-time curves from 0 h to 24 h; $V_{d_{\text{area}}}$, $V_{ss}$ - area volume of distribution, steady-state volume of distribution, respectively; MRT - mean residence time, $\text{Cl}_B$ - total body clearance; $C_{\text{max}}$ - maximum serum levels; $T_{\text{max}}$ - time of $C_{\text{max}}$; $F_{\text{abs}}$% - absolute bioavailability. $^*$ Differences are statistically significant (p<0.05).
Table 2. Integration of pharmacokinetic and pharmacodynamic data obtained for marbofloxacin after oral administration of 2 mg/kg in turkeys (n=6).

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<th>HPLC analysis</th>
<th>Microbiological assay</th>
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<tbody>
<tr>
<td>Log $E_0$ (CFU/ml)</td>
<td>-6.43</td>
<td>-6.37</td>
</tr>
<tr>
<td>Log $E_{\text{max}}$ (CFU/ml)</td>
<td>1.20</td>
<td>1.08</td>
</tr>
<tr>
<td>$E_{50}$</td>
<td>8.66</td>
<td>10.63</td>
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<tr>
<td>Log $E_{\text{max}}$ - Log $E_0$</td>
<td>7.63</td>
<td>7.45</td>
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<tr>
<td>Slope ($N$)</td>
<td>7.28</td>
<td>7.84</td>
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<thead>
<tr>
<th>Concentration$<em>{24h}$/InhibitoryActivity$</em>{\text{serum}}$</th>
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<tbody>
<tr>
<td>Bacteriostatic</td>
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<tr>
<td>Bactericidal</td>
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<tr>
<td>Elimination</td>
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Log $E_0$ - difference in log of number of bacteria (CFU/ml) in sample incubated with marbofloxacin between time 0 and 24 h, when the detection limit (10 CFU/ml) is reached; Log $E_{\text{max}}$ - difference in log of number of bacteria (CFU/ml) in control sample (absence of marbofloxacin) between time 0 and 24 h; $E_{50}$ (Concentration$_{24h}$/InhibitoryActivity$_{\text{serum50}}$) - Concentration$_{24h}$/InhibitoryActivity$_{\text{serum}}$ of drug producing 50% of the maximum antibacterial effect; $N$ - the Hill coefficient; Concentration$_{24h}$/InhibitoryActivity$_{\text{serum}}$ ratio, required for bacteriostatic, bactericidal effect and bacterial elimination.
Fig. 1. Mean serum concentrations ±SD of marbofloxacin (at a dose of 2 mg/kg, n=6) after a single i.v. (◊) or oral (▲) administration in turkeys (n=6 animals).
Fig. 2. Antibacterial activity (plots of log_{10} CFU per ml versus time) against *E. coli* O78/K80 in serum after oral administration of 2 mg/kg b.w. marbofloxacin. Values are means±SD (n=6).
Fig. 3. Plots of Concentration$_{24h}$/InhibitoryActivity$_{serum}$ versus bacterial count (log10 CFU per ml) for E. coli O78/K80 in serum of turkeys. The curve represents the line of predicted values, based on the sigmoid $E_{\text{max}}$ equation and the points are the values of the individual animals (♦ and — line – HPLC data; ◊ and - - line – microbiological data).