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Integration of Pharmacokinetic and Pharmacodynamic Indices of Marbofloxacin in Turkeys▽

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Fluoroquinolones are extensively used in the treatment of systemic bacterial infections in poultry, including systemic *Escherichia coli* bacillosis, which is a common disease in turkey flocks. Marbofloxacin has been licensed for use in various mammalian species, but not as yet 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 practice. It is generally accepted that, for fluoroquinolones, the optimal dose should be estimated on the basis of the pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of the drug under consideration. Knowledge of these specific data for the target animal species allows the establishment of 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; six animals per group) at a dose of 2 mg/kg of body weight (BW). At the same time, the serum concentrations of marbofloxacin were measured at different time intervals by a standardized high-performance liquid chromatography method, allowing the calculation of the most relevant kinetic parameters (PK parameters). The in vitro serum inhibitory activity of marbofloxacin against the selected *E. coli* strain, O78/K80, was 0.5 μg/ml in the blood serum of turkeys, and the ratio of the maximum concentration of the drug in serum to the serum inhibitory activity was 1.34. The lowest ratio of the measured serum concentration multiplied by the incubation period of 24 h to the serum inhibitory activity required for bacterial elimination was lower than the ratio of the area under the serum concentration-time curve (AUC) to the serum inhibitory activity. These first results suggested that the recommended dose of 2 mg/kg BW of 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 an AUC/MIC of >125, the generally recommended dose for all fluoroquinolones. According to the PK-PD results presented here, a dose of 3.0 to 12.0 mg/kg BW 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.

Marbofloxacin is a synthetic fluoroquinolone, developed for veterinary use only (47). It has a broad spectrum of activity (58), and bactericidal concentration-dependent killing is observed against many gram-negative bacteria (12, 49, 51, 52). The pharmacokinetic (PK) properties of marbofloxacin have been studied in several mammalian species, and some advantages over other fluoroquinolones, such as a longer elimination half-life, have been described (2, 43, 47, 48). In practice, this would enable a single treatment per 24 h, with serum concentrations remaining above the MIC for more than 12 h. Comparable kinetic data for turkeys are lacking, however, 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 the monitoring of the clinical outcomes (10, 11, 13, 14, 24, 50). The weak point of this approach is that in field trials, spontaneous clinical recovery often masks the differences in bacteriological efficacy of antibacterial drugs (Pollyanna effect), resulting in the use of suboptimal dose regimens and hence increasing the risk of resistance induction. Particularly in poultry, suboptimal antibacterial therapy comprises a risk for human health, as resistant zoonotic bacteria, like *Salmonella* spp., *Campylobacter* spp., and verotoxin-producing *Escherichia coli*, may reach the consumer (16, 29, 30, 44). Thus, therapeutic regimens need to be critically reviewed to correlate 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 PK and pharmacodynamic (PD) properties of a drug (38, 57). The integration of PK (bioavailability and clearance) and PD (MIC) indices allows the prediction of the efficacy and potency of a drug in the early phase of drug development and supports postmarketing surveillance (52, 54).

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Hence, PK-PD models serve for the selection of 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 surrogate markers for fluoroquinolones correlating with clinical cure and bacterial eradication are the area under the serum concentration-time curve (AUC)/MIC ratio and the maximum concentration of the drug in serum (C_{max})/MIC ratio (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 with calves, cows, goats, and dogs (2, 43, 48, 55). Moreover, pharmacokinetic data for marbofloxacin have been estimated for chickens and Eurasian buzzards (6, 20). However, there are no reports about PK and PK-PD indices for turkeys, and the advantages or possible disadvantages of marbofloxacin in comparison to other fluoroquinolones have not yet been evaluated.

Hence, the aim of the present study was to estimate the PK-PD surrogate markers required for bacteriostasis, bactericidal activity, and bacterial elimination, as described by Aliabadi and Lees (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 regimens.

MATERIALS AND METHODS

Drugs. Marbofloxacin (Marbocyl 10% injectable solution; Vetoquinol, batch no. 130300/1205 PdA1, V1205) was used for intravenous (i.v.) treatment. The same sterile formulation was diluted with sterile pyrogen-free water to 1% (wt/vol) and then 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 body weights of 9.9 to 10.12 kg and 6.08 to 6.96 kg, respectively. The animals were obtained from an Institute of Animal Husbandry experimental poultry farm in Stara Zagora, Bulgaria.

The 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. doses were given in the brachial vein, and the oral doses by installation of the marbofloxacin solution into the crop via a plastic tube after 12 h of food deprivation. Blood samples were collected from the brachial vein after oral administration. 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 of body weight (BW), 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 were collected prior to each treatment and at 0.25, 0.5, 0.75 (1 ml), 1, 1.5, 2, 3, 6, 9, 12, 24, 36, and 48 h (1.5 ml) after oral administration. The samples were collected without anticoagulant and kept at room temperature for 2 h in the dark. The serum samples were collected after centrifugation at 1,800 × *g* for 15 min and stored at −25°C prior to the analyses.

Determination of MIC and MBC values. (i) Bacterial isolates. The MIC and minimum bactericidal concentration (MBC) values were determined with an *Escherichia coli* strain, O78/K80, isolated from turkeys, that was obtained from the National Scientific and Diagnostic Institute of Veterinary Medicine, Sofia, Bulgaria. The *E. coli* strain was stored on beads at −20°C prior to use. *E. coli* was grown on tryptone soy blood agar (TSA; Becton Dickinson and Co., Difco Laboratories, Le Pont de Claix, France; reference 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; reference no. 275730) to obtain a turbidity comparable to the McFarland turbidity standard of 0.5. Cultures were diluted 1:100 with broth to obtain a dilution of 10^6 CFU/ml.

(ii) 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 the NCCLS [reference 41]) or to blood serum obtained from the control animals. Serial dilutions from this solution were prepared in broth and in serum with concentrations ranging between 64 μg/ml and 0.0156 μg/ml and were inoculated with approximately 5×10^5 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, the plates were incubated at 35°C overnight, and the colonies were counted. The limit of detection was 10 CFU/ml. The MIC and the serum inhibitory activity were defined as the lowest concentrations at which the bacterial growth remained below the level of the original inoculum. The MBC and the serum bactericidal activity were defined as the concentrations 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 each 0.5-ml serum sample from treated animals, 5 μl of the stationary-phase bacterial cultures was added to give a final concentration of approximately 3×10^7 CFU/ml.

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

Determination of marbofloxacin serum concentrations. (i) HPLC method. The serum concentrations of marbofloxacin were determined by high-performance liquid chromatography (HPLC) according to the method of analysis described by Garcia et al. (19). 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 (limit of quantification), and 0.01 (limit of detection) μg marbofloxacin per ml. The *r* value 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 interassay coefficients of variation (CV) for marbofloxacin were calculated to be 9.18 and 5.87, respectively.

(ii) Microbiological assay. Parallel to the HPLC determinations, the concentrations of marbofloxacin were measured by an agar-gel diffusion method using *Escherichia coli* ATCC 25922 as the 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 *r* value 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 interassay CV was 10.60. The limit of quantification in the serum samples was 0.04 μg/ml.

Pharmacokinetic analysis. Pharmacokinetic analysis of the data was performed using noncompartmental analysis based on statistical moments theory (21) (WinNonlin 4.0.1.; Pharsight Corporation, Mountain View, CA). The weighting scheme $1/y^2$ was used. The AUC was calculated by the trapezoid rule with extrapolation to infinity. The absolute bioavailability was calculated using the following equation:

$$F_{abs}\% = (AUC_{oral}/AUC_{i.v.}) \times 100 \quad (1)$$

where F_{abs} is absolute bioavailability.

Pharmacodynamic analysis. The AUC/MIC and AUC/MBC values were obtained on the basis of the area under the concentration-time curve over 24 h divided by the MIC and MBC, respectively, which were determined in broth (40). The ratio of the 24-h concentration (C_{24h}; estimated by multiplying the measured serum concentration by the incubation period of 24 h) to the serum inhibitory activity and the C_{24h}/serum bactericidal activity ratio were also determined. In these indices, the C_{24h} was divided by the serum inhibitory activity and serum bactericidal activity, respectively, as determined with serum. The C_{max}/serum inhibitory activity and C_{max}/serum bactericidal activity values were estimated by using serum inhibitory activity and serum bactericidal activity values that were determined with serum (1, 2, 3) and were used for PK-PD integration in this study. The $\log_{10}$ difference between the initial bacterial count (in number of CFU per milliliter) and the bacterial count after 24 h of incubation was also determined for turkey serum. To calculate the C_{24h}/serum inhibitory activity ratio in the effect compartment, required for determination of bacteriostatic and bactericidal activities and the total elimination of bacteria, the sigmoid inhibitory E_{max} model was used. The antibiotic response (expressed in terms of reduction of the initial bacterial count) is regressed against the surrogate marker (C_{24h}/serum inhibitory activity) by using the Hill equation:

$$E = E_{max} - [(E_{max} - E_0) \times C_e^N)/(EI_{50}^N + C_e^N)] \quad (2)$$

where *E* is the antibacterial effect measured as the change in bacterial counts (in $\log_{10}$ CFU per milliliter) in the serum sample after 24 h of incubation compared

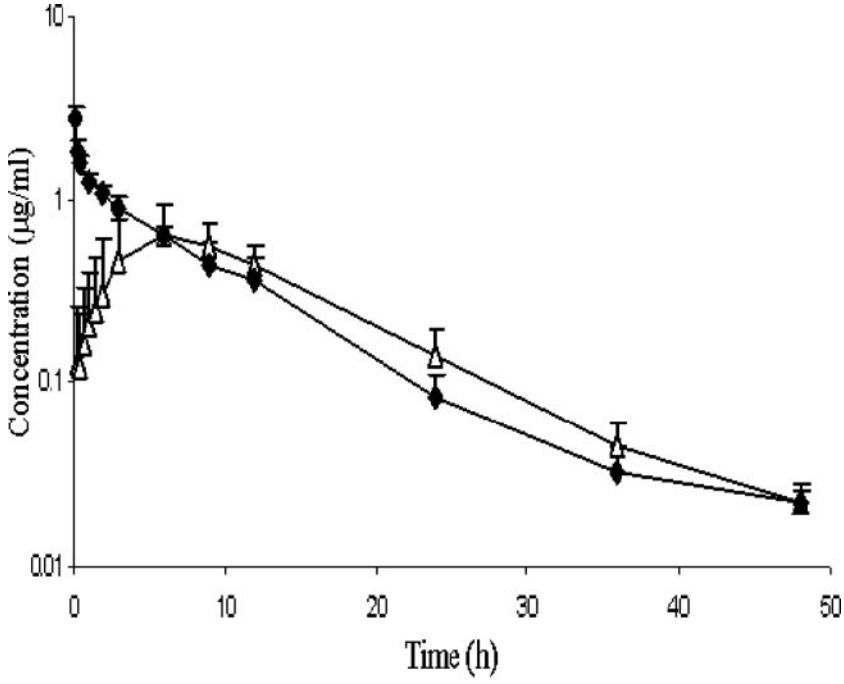

FIG. 1. Mean serum concentrations ± SD of marbofloxacin (dose, 2 mg/kg) after a single i.v. (◆) or oral (▲) administration in turkeys (n = 6 animals).

to the initial $\log_{10}$ CFU per milliliter; E_{max} is the $\log_{10}$ difference in bacterial counts between 0 and 24 h in the control sample; E_0 is the $\log_{10}$ 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; EI_{50} is the C_{24h}/serum inhibitory activity ratio producing 50% of the maximal antibacterial effect; C_e is the C_{24h}/serum inhibitory activity ratio in the effect compartment (serum); and N is the Hill coefficient, which describes the steepness of the effect curve of the C_{24h}/serum inhibitory activity ratio. In these investigations, E_{max} represents the baseline bacterial count and E_0 is the maximal effect of the drug inhibiting bacterial growth (1, 2, 5). Hence, the antibacterial response is the dependent variable representing the reduction in the initial bacterial count. The independent variable is the surrogate C_{24h}/serum inhibitory activity value. These PD indices were calculated on the basis of all samples by using the WinNonlin nonlinear regression program.

PK-PD analysis. By using in vitro MIC data and in vivo PK parameters, the surrogate markers of antimicrobial efficacy, C_{max}/MIC, AUC/MIC, and the time during which the serum concentration is greater than the MIC ($T_{>MIC}$), 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.

The antibacterial efficacy was quantified from the sigmoid E_{max} equation (equation 2) by determining the C_{24h}/serum inhibitory activity ratios 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 bacterial elimination (the lowest C_{24h}/serum inhibitory activity ratio that produced a reduction in bacterial counts to 10 CFU/ml) in serum (1).

Statistical analyses. The pharmacokinetic parameters of marbofloxacin are presented as the means ± standard deviations (SD). They were computed with the Statistica 6.1 computer program (Statistica for Windows; StatSoft, Inc., 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 P value of <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 fourfold lower than the serum inhibitory activity (0.5 μg/ml) and serum bactericidal activity (2.0 μg/ml) values.

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

Oral administration of marbofloxacin. The peak serum concentrations were found between 3 and 6 h after drug administration, and the estimated 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/serum inhibitory activity value was approximately 18 (18.4 ± 6.4), and the C_{max}/serum inhibitory activity value was 1.34 ± 0.58. The AUC/MIC (73.69 ± 25.54) and C_{max}/MIC (5.35 ± 2.31) values were nearly four times higher. The $T_{>MIC}$ value was 10.9 h.

Antibacterial activity in serum samples from animals treated orally with marbofloxacin. The activity of marbofloxacin against *E. coli* in serum samples from treated animals was determined, and a prominent inhibitory effect was observed for samples taken between 3 and 12 h, whereas at 24 and 36 h no

TABLE 1. Pharmacokinetic parameters (noncompartmental analysis) of marbofloxacin in turkeys after i.v. or oral administration of 2 mg marbofloxacin/kg BW

Method of administration	Pharmacokinetic parameter (unit)[b]	Value determined by[a]: HPLC analysis	Value determined by[a]: Microbiological assay
i.v.	$t_{1/2\beta}$ (h)	7.37 ± 1.66	9.01 ± 3.14
	CL_B (ml · h^{-1} · kg^{-1})	158.4 ± 27.5	116.6 ± 45.22
	V_{area} (liter · kg^{-1})	1.66 ± 0.34	1.75 ± 0.25
	V_{ss} (liter · kg^{-1})	1.41 ± 0.25	1.54 ± 0.19
	MRT (h)	9.04 ± 1.71	11.29 ± 3.67
	$AUC_{0\text{-}24h}$ (μg · h · ml^{-1})	11.79 ± 1.97	13.41 ± 2.64
	$AUC_{0\text{-}\infty}$ (μg · h · ml^{-1})	12.94 ± 2.21	16.71 ± 5.36[c]
Oral	MRT (h)	14.01 ± 3.38	11.69 ± 2.54
	$AUC_{0\text{-}24h}$ (μg · h · ml^{-1})	9.21 ± 3.19	10.07 ± 3.59
	$AUC_{0\text{-}\infty}$ (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_{max} (μg/ml)	0.67 ± 0.29	0.80 ± 0.32
	T_{max} (h)	6.0 ± 3.29	6.50 ± 3.51
	F_{abs} (%)	84.37 ± 21.26	70.67 ± 30.66

[a] Data represent the means ± SD of results for six individual animals.

[b] $t_{1/2\beta}$, terminal elimination half-life; $AUC_{0\text{-}\infty}$, area under the serum concentration-time curves from 0 h to ∞; $AUC_{0\text{-}24h}$, area under the serum concentration-time curves from 0 h to 24 h; V_{area}, area volume of distribution; V_{ss}, volume of distribution at steady state; MRT, mean residence time; CL_B, total body clearance; C_{max} maximum serum levels; T_{max}, time to achieve C_{max}; F_{abs}%, absolute bioavailability.

[c] Difference between assays is statistically significant ($P < 0.05$).

significant inhibition of bacteria could be measured. The antibacterial time-dependent-killing curves are presented in Fig. 2. This figure presents the control values (from serum samples taken at 0 h) showing the log-normal growth curve of the *E. coli* test strain in serum samples from untreated turkeys for comparison with the bacterial growth curves in serum samples taken at the indicated time intervals after treatment.

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

DISCUSSION

Data on the pharmacokinetics of marbofloxacin in poultry are limited, and specific pharmacokinetic data for turkeys are lacking. Hence, turkeys were treated with marbofloxacin at the recommended dose of 2 mg/kg BW, either i.v. or orally. 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 also detect any biologically

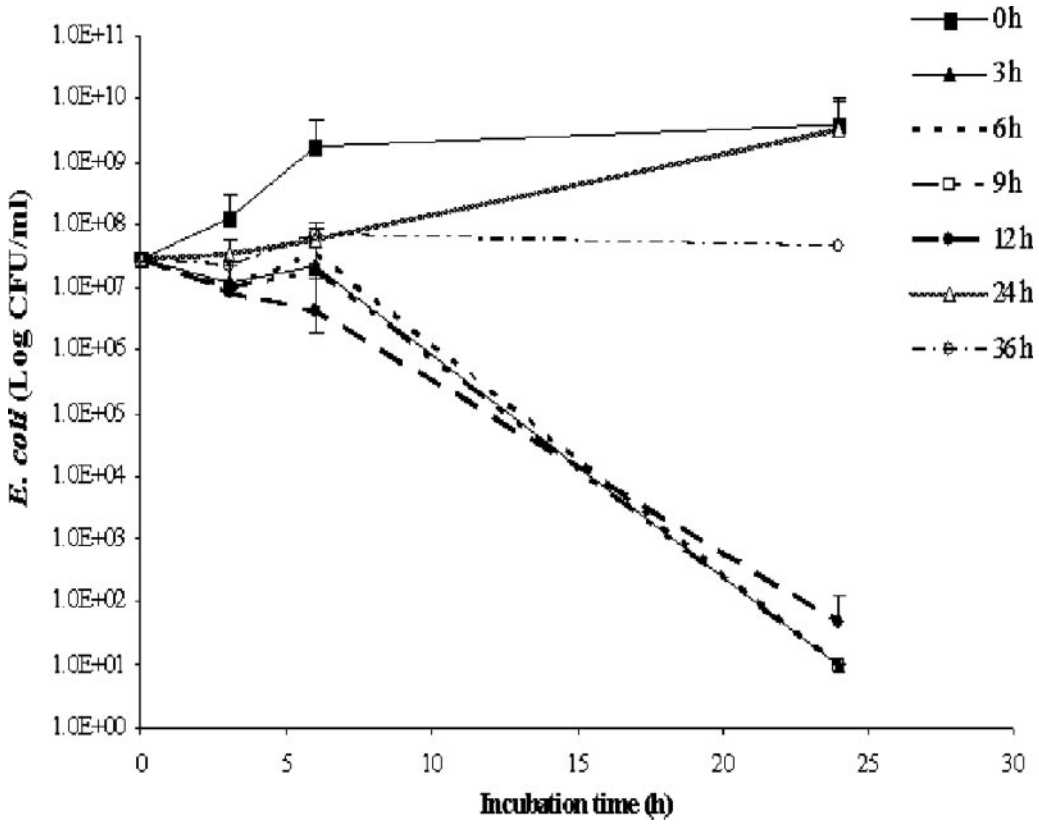

FIG. 2. Antibacterial activity (plots of $\log_{10}$ CFU per ml versus time) against *E. coli* O78/K80 in serum after oral administration of 2 mg/kg BW marbofloxacin. Values are means ± SD ($n = 6$ serum samples).

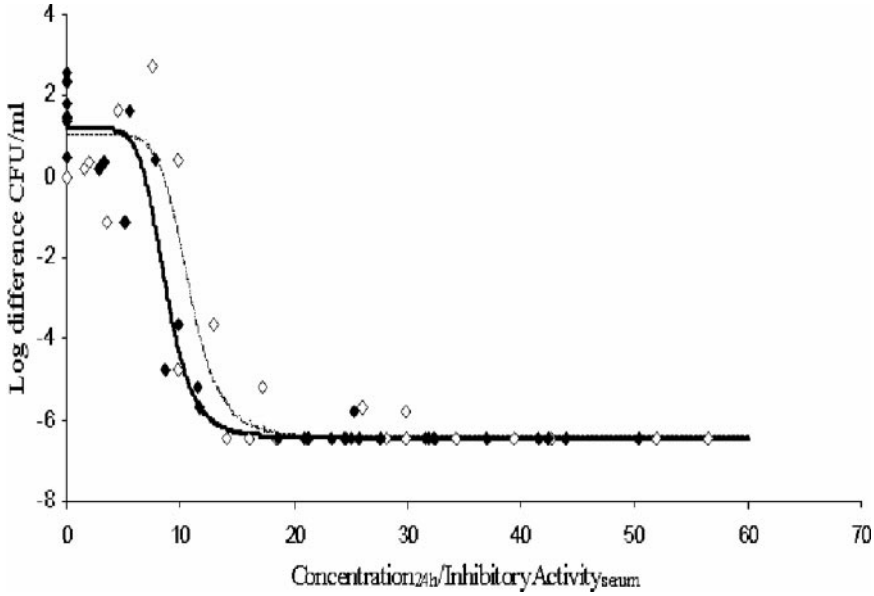

FIG. 3. Plots of C_{24h}/serum inhibitory activity values versus bacterial counts ($\log_{10}$ CFU per ml) for *E. coli* O78/K80 in serum samples from turkeys. The line represents the curve of predicted values, based on the sigmoid E_{max} equation (equation 2), and the points are the values for the individual animals (◆ and black line, HPLC data; ◇ and gray line, microbiological data).

active metabolites of marbofloxacin. The data show that the results obtained with the two assays are very comparable. This good correlation indicates that the metabolism of marbofloxacin in turkeys is limited and suggests that any formed metabolite is less microbiologically active or not active, a result that is in agreement with previous studies (6).

Following i.v. injection, the half-life at β phase ($t_{1/2\beta}$) of marbofloxacin was longer in turkeys than in broilers (5.26 h) and buzzards (4.11 h) (6, 20). In comparison with other fluoroquinolones (enrofloxacin, danofloxacin, fleroxacin, and ofloxacin), marbofloxacin has a lower volume of distribution and a longer elimination half-life (2, 7, 8, 32, 35). The calculated mean absorption time 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 lesser extent (F = 56.8%), but the C_{max} was achieved 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 marbofloxacin molecule, 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 seem to be advantageous compared to those of other fluoroquinolones (2, 7, 8, 32, 35).

TABLE 2. Integration of pharmacokinetic and pharmacodynamic data obtained for marbofloxacin after oral administration of 2 mg/kg in turkeys (n = 6)

Parameter[a]	Value determined by:	
	HPLC analysis	Microbiological assay
Indices		
Log E_0 (CFU/ml)	−6.43	−6.37
Log E_{max} (CFU/ml)	1.20	1.08
EI_{50}	8.66	10.63
Log E_{max} − Log E_0	7.63	7.45
Slope (N)	7.28	7.84
C_{24h}/serum inhibitory activities		
Bacteriostatic	6.88	8.47
Bactericidal	8.90	10.89
Bacterial elimination	12.75	15.48

[a] Log E_0, difference in log numbers of bacteria (CFU/ml) in sample incubated with marbofloxacin between time zero and 24 h, when the detection limit (10 CFU/ml) is reached; Log E_{max}, difference in log numbers of bacteria (CFU/ml) in control sample (absence of marbofloxacin) between time zero and 24 h; EI_{50}, C_{24h}/serum inhibitory activity of drug producing 50% of maximum antibacterial effect; N, the Hill coefficient; C_{24h}/serum inhibitory activity ratio, values required to achieve bacteriostatic and bactericidal effects and bacterial elimination.

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 the MIC and serum inhibitory activity values presented here are comparable to published data for the MIC_{90} values for most pathogenic *E. coli* strains (6, 49), it should be reiterated that growth curves (and MICs) measured in broth only are less representative than those determined in serum or even those from vivo findings. Our finding that in the presence of serum, the serum inhibitory activity was reduced (resulting in values that exceeded the MIC in standard broth by approximately a factor of 4) corresponds with previously reported data on the decreased antimicrobial activities of most fluoroquinolones in the presence of serum (two- to fourfold-higher MICs) (4, 5, 25, 28, 57). Protein binding explains the lower inhibitory activities of some fluoroquinolones in serum (57), but compared to other fluoroquinolones, marbofloxacin has a rather low rate of plasma

protein binding, and, hence, other factors may contribute as well to the observed differences (39).

The PK-PD indices in the current study were used according to the standardized terminology, and other terms have been defined when these indices differ from the generally accepted definitions (40). Clinical investigations in human medicine and animal studies have shown that the AUC/MIC and C_{max}/MIC ratios correlate strongly with the clinical responses to fluoroquinolones, with the first ratio having a better predictive value (42, 46). The calculated C_{max}/serum inhibitory activity values for marbofloxacin (1.34 to 1.58) were lower than 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}/serum inhibitory activity ratio obtained with the recommended therapeutic dose (6 mg/kg BW, 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 the development of antimicrobial resistance against fluoroquinolones in turkeys (17, 22, 25).

The steep slope of the curves of the C_{24h}/serum inhibitory activity ratio versus the bacterial count, with a high Hill coefficient and in vitro investigations, demonstrates 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 the $\log_{10}$ CFU/ml difference in bacterial count in the test sample containing marbofloxacin) appeared to be lower during the first 6 hours of incubation than that of danofloxacin (26). Bacterial elimination could be achieved with danofloxacin at lower C_{24h}/serum inhibitory activity ratios than with marbofloxacin (26). Marbofloxacin, however, possesses some pharmacokinetic properties that are preferable to those of other fluoroquinolones, such as low serum protein binding and total body clearance (CL_B), which should compensate for the lower activity against *E. coli* O78/K80 (25, 26).

By applying the integrated PK and PD approach and the estimated surrogate markers and by using the equation proposed by Toutain et al. (54) [dose = (AUC/MIC × CL_B × MIC)/*F*], the calculated dose for marbofloxacin equals 1.2 mg/kg BW per 24 h. Considering also the AUC/serum inhibitory activity 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 a clinical cure of *E. coli* infections. A remaining limiting variable is the variable 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 for one strain. McKellar et al. (36) suggested incorporating the MIC_{90} and MICs from one strain in the PK-PD calculation as indicative of 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 could influence the outcome of treatment, such as the immunity status of birds, physiological changes during infection, and tissue distribution of the drug, are not considered in the PK-PD modeling.

The therapeutic use of fluoroquinolones in poultry is assessed only in terms of good clinical efficacy but should 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 infections caused by gram-negative organisms, higher AUC/MIC ratios, along with high values for C_{max}/MIC and $T_{>MIC}$ surrogates, should be used to reduce the risk of resistance induction. Gunderson et al. and others recommend a breakpoint of 125 (AUC/MIC > 125) to reduce the risk of the emergence of resistance (23, 46, 57).

Following the paradigm that the AUC/MIC ratio should exceed a value of 125, with a ratio of even 400 to 500 reached under optimal conditions (27), the data presented would suggest optimal doses of 3.0 up to 12.0 mg/kg BW per day for marbofloxacin if the 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 mentioned above, the applied approach has limitations, since the activity of marbofloxacin was not determined here in challenge experiments, and the PK-PD indices serve as surrogate markers for efficacy. Therefore, clinical trials should validate this dose in diseased turkey flocks under practical conditions, not only by assessing bacteriological cure rates but also by monitoring the emergence of antibacterial resistance.

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