Comparative pharmacokinetics of chlortetracycline, tetracycline, minocycline, and tigecycline in broiler chickens

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ABSTRACT Tetracyclines continue to be important antimicrobials in veterinary medicine. However, the pharmacokinetics (**PK**) of tigecycline (**TIG**) and minocycline (**MIN**) in broiler chickens has not been investigated to date, and the PK of chlortetracycline (**CTC**) and tetracycline (**TET**) remains insufficiently researched, especially in terms of absorption. These antimicrobials have never been compared in a single setting in a single species; therefore, the aim of the present study was to compare the PK of TIG, MIN, CTC, and TET in broiler chickens. Each drug (10 mg/kg) was administered intravenously (**IV**) and orally (**PO**). The plasma concentrations of each drug were determined by liquid chromatography-tandem mass spectrometry, and the results were analyzed using compartmental and noncompartmental PK models. Despite the fact that all of the studied antimicrobials were administered at an identical IV dose, the area under the concentration–time curve between zero and the last sampling point ($AUC_{0\rightarrow t}$) for MIN (35,014 ± 3,274 μg × hour/mL) and CTC (41,851 ± 10,965 μg × hour/mL) differed significantly from that determined for TIG (18,866 ± 4,326 μg × hour/mL) and TET (17,817 ± 4,469 μg × hour/mL). After IV administration, the values of $AUC_{0\rightarrow t}$ were also directly related to total body clearance values which were significantly higher for TIG (0.56 ± 0.14 L/hour × kg) and TET (0.60 ± 0.14 L/hour × kg) than for CTC (0.25 ± 0.05 L/hour × kg) and MIN (0.29 ± 0.03 L/hour × kg). In turn, after PO administration, TIG was absorbed in only 1.55% ± 0.82, and CTC in 30.54% ± 6.99, whereas the bioavailability of MIN and TET was relatively high at 52.33% ± 3.92 and 56.45% ± 9.71, respectively. The differences in PK parameters between these drugs, despite their structural similarities, suggest that active transport mechanisms may play a role in their absorption and distribution.

Key words: tigecycline, minocycline, tetracycline, chlortetracycline, pharmacokinetics

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INTRODUCTION

In many countries, tetracycline antibiotics (**TCs**) are commonly used drugs for treating poultry (Granados-Chinchilla and Rodríguez, 2017). Certain aspects of TC use, such as typical applications, dosages, etc., have been extensively reviewed and studied (Chopra and Roberts, 2001). However, some difficulties remain when attempting to compare the pharmacokinetics (**PK**) of TCs, which constitute an important void in our knowledge. Such information enables us to understand how long a drug will be present in an organism, which has obvious implications for food production. Moreover, an understanding of how a drug's concentration in an organism changes over time allows us to predict the efficacy of different drugs, which can lead to more effective treatment protocols.

To date, most PK studies have investigated only a single TC (Anadón et al., 1985, 2012; Grabowski, 2001; Ziółkowski et al., 2016; Jasiecka-Mikołajczyk et al., 2018), whereas studies that have analyzed several agents and animal species have rarely been carried out (Ziv et al., 1974; Nielsen and Gyrd-Hansen, 1996). Studying one TC at a time has certain disadvantages because, when comparing such studies, it is difficult to account for the effects of breed, age (which is particularly important in fast growing animals like broiler chickens) (Poźniak et al., 2017), diet, pharmaceutical form of the drug (in particular, products that do not contain a

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pure substance) (Thakkar and Desai, 2015), or applied dosage (Caccia et al., 1990). Thus, the results of different experiments with the same drug, including those performed with the same species, can support different conclusions.

Therefore, the objective of the present study was to compare the PK values of 4 TCs in one species (broiler chickens) by standardizing all experimental conditions that could affect the results, including species, breed, sex, origin of animals, physiological condition, rearing conditions, as well as the dosage, form, and chemical purity of the tested drugs. To our knowledge, this is the first study to investigate the PK of tigecycline (**TIG**) and minocycline (**MIN**) in chickens, and it significantly expands on the existing knowledge of the PK of tetracycline (**TET**) and chlortetracycline (**CTC**) in this species.

MATERIALS AND METHODS

Animals

Fifty-four 3-week-old (male and female) healthy Ross broiler chickens were obtained from a commercial farm (WIMAR, Stawiguda, Poland), and were transported to the vivarium of the Faculty of Veterinary Medicine of the University of Warmia and Mazury in Olsztyn, Poland. The vivarium was air-conditioned, ambient temperature was maintained at 22°C, and relative humidity was maintained between 45 and 65%. The light cycle was identical to that applied in the commercial farm (16 h light/8 h dark). The birds were observed during a 1-week acclimatization period, and were fed the same standard broiler grower diet (drug-free) with ad libitum access to water. On the first day of the experiment, the animals were 4 wk old, and their average BW was 1.75 ± 0.19 kg. No clinical signs of disease were noted during the experiment. The birds did not receive any pharmacological treatment during the acclimatization period. The study was registered and approved by the Local Ethics Committee in Olsztyn (Ethics Committee Opinion No. 53/2018).

Experimental Design

Broilers were randomly divided into 4 intravenous (**IV**) and 4 oral (**PO**) groups. Each group consisted of 6 birds, excluding the TIG PO group which was composed of 12 broilers due to very low PO bioavailability (**F**) of TIG (Jasiecka-Mokołajczyk et al., 2018). Therefore, the number of birds in the TIG PO group was doubled to obtain the most accurate results. All animals in each group were administered selected TCs at 10 mg/kg BW. Feed was withheld for 6 h and water was withheld for 1 h before drug administration. Water was made available 1 h after and feed was made available 3 h after TC administration. The birds from the IV group received drugs into their left brachial vein via Venflon cannula, whereas animals from PO groups received TCs via a gastric tube as gavage. Animals from the PO groups were monitored for 0.5 h after drug administration for signs of regurgitation.

Blood samples of 0.4 mL each were collected into heparinized tubes from the right brachial vein with a 26 G Venflon cannula (0.6 × 20 mm) at 0 (0.083 h in IV groups, 0.16 h in PO groups, 0.25 h in IV groups, and 0.32 h in PO groups), 0.5, 1, 1, 2, 2, 3, 4, 5, 6, 8, 10, 12, 36, 48, 72, 96, and 120 h after drug administration. Plasma was separated by centrifugation at 1,650 × *g* for 10 min at 4°C and was stored at −70°C until analysis.

Chemicals and Reagents

Tigecycline hydrate (CAS 220620-09-7), CTC hydrochloride (CAS 64-72-2), TET hydrochloride (CAS 64-75-5), demeclocycline hydrochloride (CAS 64-73-3) as the internal standard (**IS**) for CTC, formic acid, acetonitrile, 1,2-dichloroethane, and water were purchased from Sigma-Aldrich (St. Louis, MO). Minocycline hydrochloride (CAS 13614-98-7) was supplied by Thermo Fisher Scientific (Waltham, MA). Tigecycline-d9 (CAS unlabeled) as the IS for TIG and MIN, and doxycycline-d3 hyclate (CAS unlabeled) as the IS for TET were purchased from Toronto Research Chemicals (North York, ON, Canada). For chromatographic analysis, the working solutions of each drug were prepared by diluting stock solutions (in methanol) in 0.1% formic acid in water.

Analytical standards of CTC, TET, and MIN dissolved in deionized water were also used for administration to animals during the PK experiment. Only TIG was not of an analytical standard due to the unavailability of TIG hydrochloride salt; therefore commercial product Tygacil (Pfizer, New York, NY) which contains a hydrochloric acid was used (TIG, lactose monohydrate, hydrochloric acid, sodium hydroxide).

Chromatography and Sample Preparation

Fully validated analytical methods were used in the Alliance 2695 HPLC system coupled with the Quattro micro API MS tandem mass spectrometer (Waters, Milford) according to the protocols described by Jasiecka-Mikołajczyk and Jaroszewski (2017) and Ziółkowski et al. (2016). The chromatographic separation of all TCs samples was performed on an Atlantis T3 column (Waters) (50 × 3 mm, 3 μm) with 3 μm particle size, at 40°C. The mobile phase consisted of 0.1% formic acid in water (phase A) and 0.1% formic acid in acetonitrile (phase B) for gradient elution set on the pump (Table 1). The run time was 6.00 min, injection volume was 1 μL for IV groups and 1.7 μL for PO groups, and the autosampler temperature was 4°C. Detection was performed in positive ion mode in multiple reaction monitoring, according to the parameters in Table 1.

Plasma samples for the determination of TIG and MIN were prepared based on the analytical method of Jasiecka-Mikołajczyk and Jaroszewski (2017) with minor modifications (TIG-d9 was the IS, and a shorter

Table 1. Parameters of HPLC coupled with tandem mass spectrometer.

Parameters		Compound						
		TIG	MIN	TIG-d9	CTC	DMC	TET	DOX-d3
Precursor ions (m/z)		293.60	458.0	298.60	479.15	465.10	445.15	448.20
Product ions (m/z)		257.10	441.20	290.10	462.15	448.05	410.50	431.20
Desolvation gas		Nitrogen						
Desolvation gas temperature (°C)		350			350	350	390	390
Desolvation gas flow (L/hour)		800						
Cone gas flow (L/hour)		200			200	200	50	
Collision gas		Argon						
Source temperature (°C)		120						
Electrospray mode		Positive						
Cone voltage (V)		18	35	17	26	30	30	30
Capillary voltage (kV)		3.30	3.30	3.30	3.30	3.30	3.30	3.30
Collision energy (eV)		12	22	11	18	13	20	20
Time (min)	Mobile phase							
0	A%	95.0	95.0	95.0	95.0	95.0	80.0	80.0
	B%	5	5	5	5	5	20	20
1.33 (1.66 CTC)	A%	50	50	50	75	75	0	0
	B%	50	50	50	25	25	100	100
2.00	A%	0	0	0	0	0	0	0
	B%	100	100	100	100	100	100	100
2.66	A%	95.0	95.0	95.0	95.0	95.0	80	80
	B%	5	5	5	5	5	20	20
6.00	A%	95.0	95.0	95.0	95.0	95.0	80	80
	B%	5	5	5	5	5	20	20
Flow rate		0.45 mL/min						
Phase A		0.1% formic acid in water						
Phase B		0.1% formic acid in acetonitrile						
Column		Atlantis T3 (3 μm; 3.0 × 50 mm)						
Column temperature		40°C						
Autosampler temperature		4°C						
Injection volume		1.0 μL for intravenous/1.7 μL for oral administration						

Abbreviations: CTC, chlortetracycline hydrochloride; DMC, demeclocycline hydrochloride (as internal standard for CTC); DOX-d3, deuterium-labeled doxycycline (as internal standard for TET); MIN, minocycline hydrochloride; TIG, tigecycline hydrate; TIG-d9, deuterium-labeled tigecycline (as internal standard for TIG and MIN); TET, tetracycline hydrochloride.

column was used). Moreover, for CTC and TET analysis the sample preparation method described by Ziółkowski et al. (2016) was used, however, with significant modifications (CTC and TET were used instead of oxytetracycline). Therefore, the mentioned method had to be revalidated in terms of calibration, quality control (**QC**) points, total recovery, and matrix effect steps. The calibration curves for IV groups comprised 10 points within a concentration range of 0.05 to 50.0 μg/mL. The calibration curves for PO groups comprised 11 points within a concentration range of 0.01 to 10.0 μg/mL. Four points in each calibration type were used as QC: low QC, intermediate QC, medium QC, and high QC.

The revalidated analytical method for the determination of TET and CTC was characterized by high linearity. The coefficient of correlation r^2 was ≥ 0.99 for all calibration curves, and back-calculated concentrations at all points deviated from the normal value by ± 15%. The method's accuracy was determined at 2.0 to 9.0% based on the differences between QC, and precision ranged from 3.0 to 8.0% based on the values of the coefficient of variation for QC. In the developed method, total recovery was estimated at 60% for TET and 52% for CTC. Total recovery was around 38% for the IS. The matrix effect was verified by analyzing the signal intensity at QC in water and in plasma as the matrix. The mean value of this parameter was 3% for TET and 7% for CTC.

Pharmacokinetic Analysis

Mean plasma concentrations vs. time data were analyzed using the ThothPro commercial software program (ThothPro, Gdańsk, Poland). Data were fitted to a two-compartment model for IV administration and a one-compartment model for PO administration based on the lower value of the Akaike information criterion (Yamaoka et al., 1978). A non-compartmental analysis was additionally performed for both routes of administration.

The following parameters were examined in the PK compartmental analysis of both routes of administration:

the area under the concentration–time curve calculated for the concentration range of zero to infinity ($AUC_{0\rightarrow\infty}$) and zero to t ($AUC_{0\rightarrow t}$) according to the linear trapezoidal rule; the residual part of the area under the curve ($AUC_{rest\%}$) expressed as % of $AUC_{0\rightarrow\infty}$; a mathematical coefficient of plasma concentration extrapolated to time zero of the elimination phase (A_2); the slope of the elimination phase (β) (the elimination rate constant which in the one-compartmental analysis is equal to the rate constant from compartment 1 to zero and/or k_{el}); and half-life in the elimination phase ($t_{1/2\beta}$). Additionally, using the non-compartmental analysis, the mean residence time from zero to t ($MRT_{0\rightarrow t}$) and from zero to infinity ($MRT_{0\rightarrow\infty}$) was calculated based on $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$, and the area under the first moment curve from zero to t ($AUMC_{0\rightarrow t}$) and zero to infinity ($AUC_{0\rightarrow\infty}$).

The following parameters in PK compartmental analysis were also determined only in IV groups: a mathematical coefficient of plasma concentration extrapolated to time zero of the distribution phase (A_1); the slope of the distribution phase (distribution rate constant) (α); half-life in the distribution phase ($t_{1/2\alpha}$); the overall rate constant for drug elimination from the central compartment (1) (rate constant from compartment 1 to zero) (k_{10}); the rate constant for drug elimination from the peripheral compartment (2) at any time (rate constant from compartment 2 to zero) (k_{20}); the first-order distribution rate constant between compartment 2 and compartment 1 (k_{21}); the first-order distribution rate constant between compartment 1 and compartment 2 (k_{12}); central (Vd_c) and peripheral ($Vd_{peripheral}$) volume of distribution; and total body clearance (Cl_B). Additionally, using the non-compartmental analysis, the apparent volume of distribution (Vd_{area}) based on $AUC_{0\text{-}t}$ and volume of distribution at steady state (Vd_{ss}) were calculated.

In all PO groups, the absorption rate constant (k_{ab}) was determined from the slope of β according to the one-compartmental analysis. This parameter was used to calculate the mathematical coefficient of plasma concentration extrapolated to time zero of the absorption phase (A_3). The mean absorption time (**MAT**) and half-life in the absorption phase ($t_{1/2kab}$) were calculated according to Gibaldi and Perrier (1982):

$$MAT = \frac{1}{k_{ab}}$$

$$t_{1/2kab} = \frac{0.693}{k_{ab}}$$

The observed values of the maximum and the last plasma concentrations (C_{max} and C_{last}, respectively) and the time of C_{max} and C_{last} (t_{max} and t_{last}, respectively) after PO administration of the drugs were determined individually for each animal and were expressed as mean values (±SD). In turn, C_{max} denoted the first concentration ($C_{0.083}$) determined at time t_{max} ($t_{0.083}$) after IV administration, and both parameters were also expressed as mean values (±SD). The value of F was calculated using the following equation (Ziółkowski et al., 2014):

$$F = \frac{AUC_{0\rightarrow t_{POindividual}}}{AUC_{0\rightarrow t_{IVmean}}} \times 100\%$$

Statistical Analysis

For each method of drug administration (PO, IV), the differences between TCs values with regard to plasma concentrations and PK parameters were assessed using one-way independent ANOVA followed by multiple comparisons between groups using the Holm-Sidak method. These calculations were performed using SigmaPlot v. 12.0 (Systat Software, San Jose, CA). Results are expressed as arithmetic means ± SD. Differences were regarded as statistically significant at $P < 0.05$.

RESULTS

TCs Concentrations

After IV administration, the plasma concentrations of MIN were significantly higher than those of the other 3 TCs for the first 2.5 h (Figure 1; Supplementary Tables 1, 2). The plasma concentrations of TIG and TET tended to be similar and generally lower than those of the other TCs. Specifically, MIN concentrations were significantly different from CTC concentrations except at 0.25 h ($P = 0.99$), 3 h ($P = 0.508$), and 4 h ($P = 0.062$). Minocycline concentrations were at least twice as high as TIG concentrations between 0.25 and 5 h, and twice as high as those of TET between 0.083 and 4 h. Concentrations of CTC were at least twice as high as those of TIG except at 0.083, 24, and 36 h, and at least twice as high as those of TET at all time points except 0.5 h ($P = 0.061$). Tigecycline and TET concentrations only differed significantly at 0.083 h.

Similar to the results of IV administration, the plasma concentrations of MIN after PO administration were also significantly higher than those of the other TCs at nearly all sampling times (Figure 1; Supplementary Tables 3, 4). In contrast, TIG plasma concentrations were at least 20-fold lower than those of CTC, TET, and MIN at all sampling times. Tigecycline was detectable only 13.5 ± 5.05 h after drug administration, whereas CTC, TET, and MIN were detectable for much longer (66.0 ± 14.70 h, 88.0 ± 12.39 h, and 96.0 h, respectively). The concentrations of TET and CTC were similar at the beginning of the experiment, and then after 4.0 h, they differed significantly.

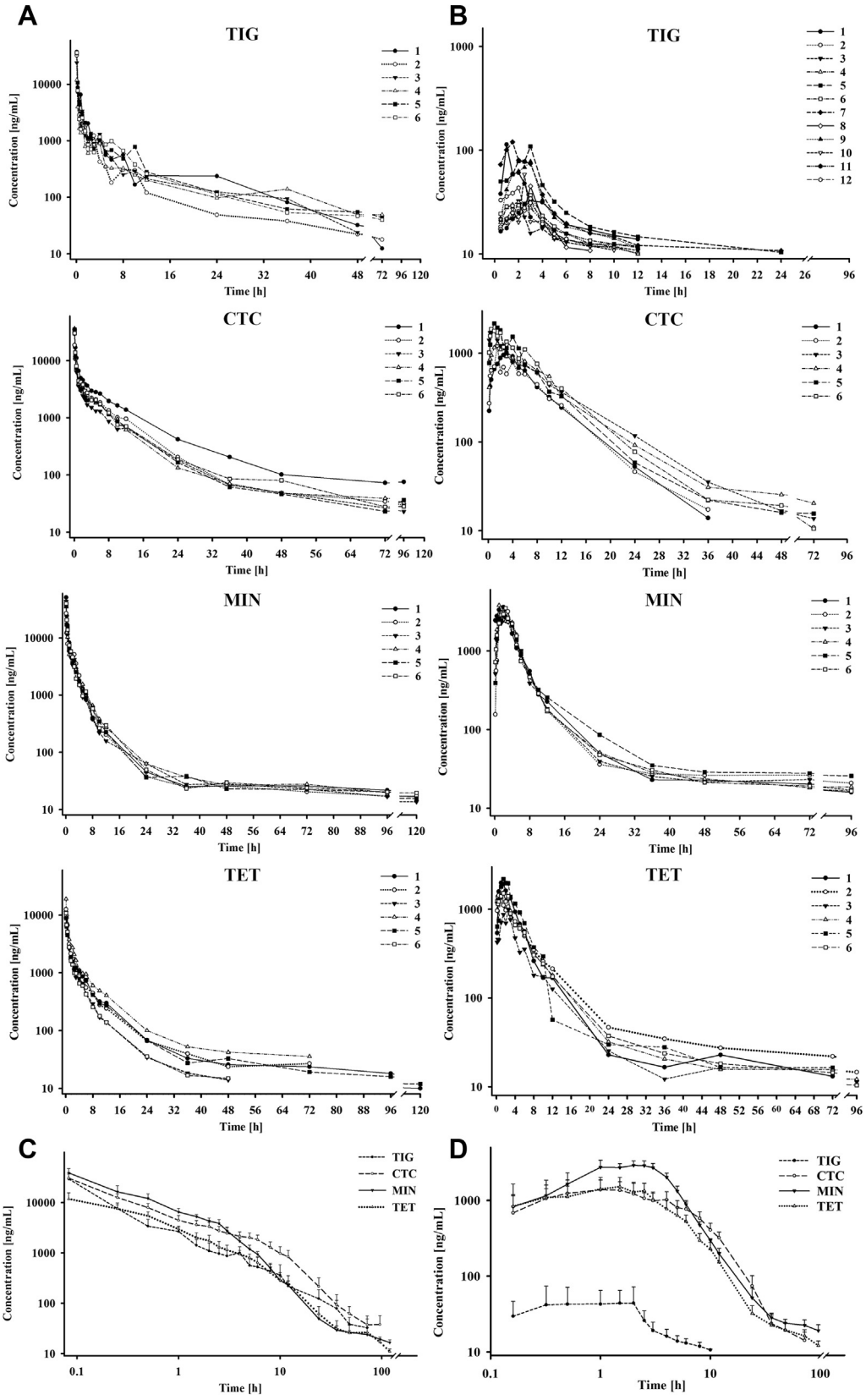

Figure 1. Individual (semi-log plot: A: intravenous; B: oral) and mean (log-log plot: C: intravenous; D: oral) plasma concentration–time profiles of tigecycline (TIG), chlortetracycline (CTC), minocycline (MIN), and tetracycline (TET) administered to broiler chickens at a dose of 10 mg/kg BW.

Pharmacokinetics after IV Administration

The PK parameters of TCs after IV administration differed across the analyzed drugs, but no significant differences in MRT or k_{20} were observed between the tested agents (Table 2). Chlortetracycline and MIN were most similar in PK parameters, and the only differences were noted in the values of k_{12} and $AUMC_{0\text{-}t}$ which were higher in CTC, and the values of Vd_{area} which were lower in CTC than in MIN.

However, greater differences in PK parameters were observed in some cases. The values of α, β, k_{12}, k_{21}, and Vd_{ss} were at least 1.5-fold higher in TIG, whereas $t_{1/2\alpha}$, $t_{1/2\beta}$, AUC, and Cl_B were at least 1.5-fold lower in TIG than in MIN and CTC. The values of AUMC in CTC and Vd_{area} in MIN were more than 1.5-fold higher compared with TIG, whereas the value of A_2 was nearly 5 times higher in TIG than in MIN. Tigecycline and TET were also similar in the values of AUC, AUMC, Cl_B, Vd_{area}, Vd_{ss}, $t_{1/2\beta}$, and β, whereas the remaining parameters differed significantly in the compared drugs. The values of A_1, A_2, α, k_{10}, k_{12}, and k_{21} were at least twice higher in TIG than in TET, whereas distribution parameters such as $t_{1/2\alpha}$, Vd_{area}, and Vd_c were significantly lower in TIG than in TET (Table 2; Supplementary Table 5).

Only several similarities were found between TET and CTC (in the values of A_2, β, k_{10}, k_{21}, and $t_{1/2\beta}$) and between TET and MIN (in the values of A_2, β, k_{10}, k_{21}, k_{12}, AUMC, and Vd_{area}). In turn, the values of A_1, α, and AUC in TET were twice (or more) lower, and the values of $t_{1/2\alpha}$, Cl_B, Vd_{ss}, and Vd_c in TET were 1.5-fold (or more) higher compared with CTC and MIN. Tetracycline was characterized by higher values of k_{12} and AUMC and a 1.5-fold lower value of Vd_{area} in comparison with CTC, whereas the value of $t_{1/2\beta}$ in TET was nearly half of that noted in MIN (Table 2; Supplementary Table 5).

Pharmacokinetics after PO Administration

In the group of the studied agents, TIG was absorbed from gastrointestinal tract 20 times less than CTC, 33 times less than MIN, and 37 times less than TET, as

Table 2. Mean (±SD) value of selected pharmacokinetic parameters (2- and non-compartmental analysis) of tetracyclines after intravenous administration to broiler chickens (n = 6) at a dose of 10 mg/kg.

Pharmacokinetic parameters	Tigecycline	Chlortetracycline	Minocycline	Tetracycline
Two-compartmental				
A_1 (ng/mL)	37,241.76 ± 13,190.79[a]	34,316.54 ± 7,133.43[a]	41,356.94 ± 11,035.01[a]	12,867.01 ± 3,863.02[b]
A_2 (ng/mL)	240.49 ± 131.02[a]	132.51 ± 76.71[a,b]	54.64 ± 9.02[b]	78.30 ± 9.87[b]
α (h^{-1})	5.19 ± 0.79[a]	3.12 ± 0.60[b]	2.69 ± 0.48[b]	1.78 ± 0.30[c]
β (h^{-1})	0.03 ± 0.01[a]	0.01 ± 0.003[b]	0.01 ± 0.001[b]	0.02 ± 0.01[a,b]
k_{10} (h^{-1})	2.57 ± 0.90[a]	1.74 ± 0.47[a,b]	1.96 ± 0.37[a,b]	1.14 ± 0.25[b]
k_{20} (h^{-1})	0.06 ± 0.02	0.03 ± 0.01	0.04 ± 0.01	0.08 ± 0.06
k_{21} (h^{-1})	0.07 ± 0.04[a]	0.03 ± 0.01[b]	0.01 ± 0.001[b]	0.03 ± 0.01[b]
k_{12} (h^{-1})	2.58 ± 0.60[a]	1.37 ± 0.22[b]	0.72 ± 0.23[c]	0.63 ± 0.21[c]
$t_{1/2\alpha}$ (h)	0.14 ± 0.02[a]	0.23 ± 0.05[b]	0.27 ± 0.06[b]	0.40 ± 0.06[c]
$t_{1/2\beta}$ (h)	25.17 ± 8.59[a]	54.03 ± 13.05[b,c]	70.61 ± 4.95[b]	39.04 ± 18.71[a,c]
$t_{0.083}$ (h)	0.083	0.083	0.083	0.083
t_{last} (h)	64.0 ± 12.39	96	120	80 ± 32.79
$C_{0.083}$ (ng/mL)	29,523.63 ± 9,897.74[a]	30,638.03 ± 6,550.20[a]	38,901 ± 8,169.16[a]	11,827.95 ± 3,757.69[b]
C_{last} (ng/mL)	31.08 ± 15.08	37.57 ± 19.23	18.03 ± 4.19	18.87 ± 10.08
$AUC_{0\rightarrow t}$ (μg × hour/mL)	18,866.22 ± 4,326.76[a]	41,851.65 ± 10,965.45[b]	35,014.37 ± 3,274.26[b]	17,817.48 ± 4,469.50[a]
$AUC_{0\rightarrow\infty}$ (μg × hour/mL)	20,144.50 ± 4,336.36[a]	44,547.91 ± 12,230.36[b]	36,730.17 ± 3,263.02[b]	18,996.71 ± 5,584.39[a]
$AUC_{rest\%}$	6.54 ± 3.01	5.83 ± 1.68	4.70 ± 1.11	5.36 ± 3.66
Cl_B (L/hour × kg)	0.56 ± 0.14[a]	0.25 ± 0.05[b]	0.29 ± 0.03[b]	0.60 ± 0.14[a]
Vd_c (L/kg)	0.31 ± 0.17[a]	0.31 ± 0.09[a]	0.25 ± 0.06[a]	0.85 ± 0.23[b]
$Vd_{peripheral}$ (L/kg)	12.68 ± 4.16	17.43 ± 5.40	13.79 ± 6.33	18.18 ± 9.81
Non-compartmental				
$AUMC_{0\rightarrow t}$ (μg × hour/mL^{-2})	163,621.70 ± 45,390.76[a]	429,213.52 ± 169,611.74[b]	241,424.07 ± 21,106.43[a]	147,432.01 ± 73,497.20[a]
$AUMC_{0\rightarrow\infty}$ (μg × hour/mL^{-2})	292,857.78 ± 98,835.76[a]	905,368.79 ± 398,903.27[b]	683,283.47 ±104,920.76[a,b]	329,996.67 ± 255,809.87[a]
$MRT_{0\rightarrow t}$ (h)	8.68 ± 1.51	10.04 ± 1.09	6.92 ± 0.67	7.97 ± 3.27
$MRT_{0\rightarrow\infty}$ (h)	14.56 ± 3.32	19.79 ± 3.88	18.58 ± 3.04	15.51 ± 8.05
Vd_{area} (L/kg)	20.10 ± 7.70[a]	19.50 ± 6.18[a]	32.40 ± 4.72[b]	31.04 ± 9.52[b]
Vd_{ss} (L/kg)	4.84 ± 1.70[a]	2.47 ± 0.36[b]	2.00 ± 0.32[b]	4.68 ± 1.84[a]
AIC				
One- compartment model	334.46 ± 20.56	345.13 ± 7.71	369.90 ± 8.22	303.69 ± 18.78
Two-compartment model	283.0 ± 23.87	297.44 ± 7.92	322.50 ± 7.27	245.41 ± 20.48

[a–c]Significant differences ($P < 0.05$) in a row between values of pharmacokinetic parameters among tetracyclines.

A_1 and A_2, mathematical coefficients, plasma concentrations extrapolated to time zero of the first/distribution and second/elimination phases, respectively; α, slope of distribution (initial) of the phase/distribution rate constant; β, slope of the second (post-distribution/terminal/elimination) phase/post-distribution rate constant; k_{10}, overall rate constant for drug elimination by the central compartment (1) at any time = pure elimination rate constant = rate constant from compartment 1 to zero; k_{20}, rate constant for drug elimination by the peripheral compartment (2) at any time = rate constant from compartment 2 to zero; k_{21}, first order distribution rate constant between the peripheral (2) and the central compartment (1); k_{12}, first order distribution rate constant between the central (1) and the peripheral compartment (2); $t_{1/2\alpha}$, half-life in distribution (α) phase; $t_{1/2\beta}$, half-life in elimination (β) phase; $t_{0.083}$, time of first measure concentration; t_{last}, time of last measured concentration; $C_{0.083}$, first measure plasma concentration; C_{last}, last measured plasma concentration; $AUC_{0\rightarrow t}$, area under the concentration vs. time curve from zero to t; $AUC_{0\rightarrow\infty}$, area under the concentration vs. time curve from zero to infinity; $AUC_{rest\%}$, residual observed part of the area under the curve; $AUMC_{0\rightarrow t}$, area under the first moment of curve from zero to t; $AUMC_{0\rightarrow\infty}$, area under the first moment of curve from zero to infinity; Cl_B, total body clearance; $MRT_{0\rightarrow t}$, mean residence time from zero to t; $MRT_{0\rightarrow\infty}$, mean residence time from zero to infinity; Vd_{area}, apparent volume of distribution; Vd_{ss}, volume of distribution in steady state; Vd_c, volume of distribution of central compartment; $Vd_{peripheral}$, volume of distribution of peripheral compartment; AIC, Akaike information criterion.

demonstrated by the differences in the values of F and, indirectly, also in C_{max}, AUC, and A_3 (Table 3; Supplementary Table 6). In addition, CTC was absorbed more than 1.5 times less than MIN and TET, whose F was similar but AUC of MIN was twice as high as TET. Nevertheless, despite the differences in the absorption, the time of this process in all TCs was similar, as demonstrated by similar values of $t_{1/2kab}$, MAT, and k_{ab}. Differences in the absorption also affected the elimination phase, and the evaluated TCs were arranged in the following order based on the values of $t_{1/2\beta}$: TIG < CTC < TET < MIN. However, β was the only parameter that was higher in TIG (5-fold) than in MIN and TET (Table 3; Supplementary Table 6).

DISCUSSION

The results of our study indicate that, in broiler chickens, plasma concentrations of TIG, MIN, TET, and CTC differ even when these antimicrobials are administered in the same fashion, at the same dose, and under identical conditions (Figure 1). However, after IV administration, the mean residence times of all 4 drugs are similar. After PO administration, only the mean residence time of TIG differs markedly from those of the other drugs, and the MAT of all 4 drugs are similar. Our results also indicate that, after IV administration, the AUC values of MIN and CTC are more than twice as large as those of TIG and TET (Figure 1, Table 2). Finally, our results indicate that the F of TIG is much lower than that of the other tetracyclines.

As would be expected, given the differences in experimental design, the results of our study differ to a greater or lesser extent from those of other studies of the PK of TCs in poultry. In general, the PK of TIG in our chickens was similar to that in turkeys (Jasiecka-Mikołajczyk et al., 2018), although the F was even lower in turkeys.

The $t_{1/2\beta}$ of MIN in our chickens was more than 10-fold higher than that previously reported for hens and turkeys (Grabowski, 2001). However, this difference could be due to the higher dose and the more sensitive analytical method used in our study, as the sampling time in our study was 120 h, whereas that used by Grabowski (2001) was 48 h.

Here, we provide the first report of the F value of PO-administered TET in chickens. With regard to IV TET administration, our $t_{1/2\alpha}$ value was 2-fold lower, and our $t_{1/2\beta}$, Cl_B, and V_c values were 20-, 6-, and 20-fold higher than those reported by Anadón et al. (1985). These differences in PK parameters are likely due to differences in experimental design, including breed, age, drug dose, sampling time, and drug concentrations.

In our study, the $t_{1/2\beta}$ of TET was more than 7 times longer than that reported by Pollet et al. (1983) and Anadón et al. (2012). This difference is likely due to the very sensitive analytical method we used, which enabled a sampling time of 96 h. Our F value was nearly twice as high as that reported by Anadón

Table 3. Mean (±SD) value of selected pharmacokinetic parameters (one- and non-compartmental analysis) of tetracyclines after oral administration to broiler chickens (tigecycline n = 12, others n = 6) at a dose of 10 mg/kg.

Pharmacokinetic parameters	Tigecycline	Chlortetracycline	Minocycline	Tetracycline
One-compartmental				
A_2 (ng/mL)	21.87 ± 4.48	224.31 ± 401.70	44.15 ± 14.93	38.08 ± 9.75
A_3 (ng/mL)	7.81 ± 11.92[a]	612.03 ± 480.93[b]	899.39 ± 746.78[b]	768.34 ± 293.71[b]
β (h^{-1})	0.054 ± 0.03[a]	0.038 ± 0.04[a,b]	0.01 ± 0.002[b]	0.01 ± 0.001[b]
k_{ab} (h^{-1})	1.59 ± 1.77	1.08 ± 0.58	0.91 ± 0.55	0.70 ± 0.52
$t_{1/2\beta}$ (h)	15.13 ± 6.11[a]	30.59 ± 18.01[b]	80.89 ± 16.84[c]	58.0 ± 6.64[d]
$t_{1/2kab}$ (h)	0.84 ± 0.52	0.99 ± 0.93	1.19 ± 1.12	1.85 ± 1.50
t_{max} (h)	2.38 ± 0.68	1.42 ± 0.86	2.08 ± 0.67	1.42 ± 0.67
t_{last} (h)	13.5 ± 5.05	66.0 ± 14.70	96.0	88.0 ± 12.39
C_{max} (ng/mL)	60.83 ± 35.06[a]	1,560.8 ± 450.04[b]	3,227.7 ± 506.03[c]	1,606.63 ± 422.08[b]
C_{last} (ng/mL)	11.21 ± 1.04[a]	14.15 ± 3.63[a]	19.1 ± 3.69[b]	12.98 ± 2.22[a]
$AUC_{0\to t}$ (μg×hour/mL)	292.0 ± 154.95[a]	12,780.81 ± 2,923.61[b]	18,322.26 ± 1,370.90[c]	10,057.10 ± 1,729.45[d]
$AUC_{0\to\infty}$ (μg×hour/mL)	534.11 ± 228.38[a]	13,415.51 ± 3,156.40[b]	20,426.19 ± 1,552.84[c]	11,130.86 ± 1,806.97[d]
$AUC_{rest\%}$	46.16 ± 9.76[a]	4.47 ± 3.74[b]	10.27 ± 2.04[b]	9.82 ± 1.96[b]
MAT (h)	1.22 ± 0.76	1.43 ± 1.34	1.73 ± 1.62	2.67 ± 2.16
F (%)	1.55 ± 0.82[a]	30.54 ± 6.99[b]	52.33 ± 3.92[c]	56.45 ± 9.71[c]
Non-compartmental				
$AUMC_{0\to t}$ (μg×hour/mL^{-2})	1,576.79 ± 1,274.82[a]	120,624.7 ± 32,192.34[b]	177,687.34 ± 20,532.66[c]	108,713.51 ± 24,543.63[b]
$MRT_{0\to t}$ (h)	5.07 ± 1.16[a]	9.44 ± 1.32[b]	9.74 ± 1.05[b]	11.10 ± 2.66[b]
AIC				
One- compartment model	72.04 ± 21.99	233.35 ± 13.20	294.97 ± 9.21	252.57 ± 25.99
Two-compartment model	126.54 ± 43.92	242.59 ± 25.15	313.19 ± 26.10	261.98 ± 30.10

[a–d]Significant differences ($P < 0.05$) in a row between values of pharmacokinetic parameters among tetracyclines.

A_2, mathematical coefficients, plasma concentrations extrapolated to time zero of the second/elimination phase; A_3, mathematical coefficients for the absorption phase; β, slope of the second (post-distribution/terminal/elimination) phase/post-distribution rate constant (in one-compartmental analysis $\beta = k_{10}$, overall rate constant for drug elimination by the central compartment (1) at any time = pure elimination rate constant = rate constant from compartment 1 to zero); $t_{1/2\beta}$, half-life in elimination (β) phase; k_{ab}, absorption rate constant; $t_{1/2kab}$, half-life in absorption phase; t_{max}, time of maximum concentration; t_{last}, time of last measured concentration; C_{max}, maximum plasma concentration; C_{last}, last measured plasma concentration; $AUC_{0\to t}$, area under the concentration vs. time curve from zero to t; $AUC_{0\to\infty}$, area under the concentration vs. time curve from zero to infinity; $AUC_{rest\%}$, residual observed part of the area under the curve; $AUMC_{0\to t}$, area under the first moment of curve; $MRT_{0\to t}$, mean residence time; MAT, mean absorption time; F, absolute bioavailability; AIC, Akaike information criterion.

et al. (2012). However, it may not be appropriate to compare these values, as we used the same dose of TET (10 mg/kg) for both PO and IV administration, whereas Anadón et al. (2012) used doses of 60 and 15 mg/kg. The PK of TCs is dose-dependent (Adir and Barr, 1978), which suggests that the value of F may not have been accurately determined by Anadón et al. (2012).

Interestingly, we found that the F values of these TCs differ considerably. This has also been observed in studies on other species, including humans (Supplementary Table 7). In our opinion, the structures of these substances are so similar that differences in their rates of diffusion across membranes cannot account for all of the differences in F values. We speculate that, instead, absorption of these drugs from the gastrointestinal tract and their distribution across biological barriers could be regulated by active cell transport mechanisms like efflux pumps. This hypothesis could also explain the differences in other PK parameters, such as AUC, Cl_B, $t_{1/2\alpha}$, $t_{1/2\beta}$, and V_d after IV administration.

In conclusion, our study indicates that, despite similarities in their structure and physicochemical properties, the PK of TIG, MIN, TET, and CTC differ substantially, particularly with regard to plasma concentrations, AUC, and F. These differences suggest that active transport mechanisms, such as efflux pumps, may play an important role in the absorption and distribution of these drugs.

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.psj.2020.06.038

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