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PHARMACOKINETICS AND BIOAVAILABILITY OF DOXYCYCLINE IN FASTED AND NONFASTED BROILER CHICKENS

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The pharmacokinetics and the influence of food on the kinetic profile and bioavailability of doxycycline was studied after a single intravenous (i.v.) and oral dose of 10.0 mg/kg body weight in 7-week-old broiler chickens. Following i.v. administration the drug was rapidly distributed in the body with a distribution half-life of 0.21 ± 0.01 h. The elimination half-life of 6.78 ± 0.06 h was relatively long and resulted from both a low total body clearance of 0.139 ± 0.007 L/h·kg and a large volume of distribution of 1.36 ± 0.06 L/kg. After oral administration to fasted chickens, the absorption of doxycycline was quite fast and substantial as shown by the absorption half-life of 0.39 ± 0.03 h, the maximal plasma concentration of 4.47 ± 0.16 μg/mL and the time to reach the C_{max} of 1.73 ± 0.06 h. The distribution and the final elimination of the drug were slower than after i.v. administration. The absolute bioavailability was 73.4 ± 2.5%. The presence of food in the intestinal tract reduced and extended the absorption ($t_{1/2a}$ = 1.23 ± 0.21 h; C_{max} = 3.07 ± 0.23 μg/mL; t_{max} = 3.34 ± 0.21 h). The absolute bioavailability was reduced to 61.1% ± 4.4%.

Key words: Doxycycline, pharmacokinetics, bioavailability, chicken, food

Doxycycline is a semisynthetic tetracycline derivative. It is 5 to 10 times more lipid soluble than the traditional tetracyclines, providing marked advantages in terms of a more extensive absorption after oral administration, a wider distribution and a greater tissue penetration, a prolonged biological half-life, as well as an enhanced antibacterial activity against several organisms (Aronson, 1980; Shaw and Rubin, 1986). As regards the avian species, the pharmacokinetics and bioavailability of doxycycline have been extensively studied in turkeys (Küng and Wanner, 1994; Santos et al., 1996; Santos et al., 1997). Much less information is available on the pharmacokinetics of the drug in chickens (Anadón et al., 1994), and there are no data on the possible influence of food on these

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properties. In turkeys, the bioavailability of doxycycline was dramatically affected by the presence of food in the intestinal tract (Santos et al., 1996). In human beings and mammals, however, doxycycline absorption was found to be much less affected by food (Welling et al., 1977; Meijer et al., 1993).

The present studies were undertaken to study the pharmacokinetic profile and the influence of food on the pharmacokinetics and bioavailability of doxycycline in broiler chickens, after a single i.v. and oral administration, respectively.

Materials and methods

Animals and housing

Eighteen healthy 7-week-old Arbor Acres broiler chickens of both sexes were used in the experiment. The animals were purchased from a poultry farm and housed in individual cages in an experimental animal house. The mean body weight (± SD) of the birds was 2.04 ± 0.10 kg. Before the commencement of the experiment the animals were acclimatised for 2 weeks. The room temperature was 20–22 °C and the relative humidity was maintained at 50–70%. Commercial diet and water were provided *ad libitum*. The ration did not contain any drug or growth promoter.

Experimental design

Birds were allotted to three groups with six animals in each. Chickens of Group 1 were given doxycycline intravenously, whereas to Groups 2 and 3 the drug was administered orally, in each case at a dose of 10 mg/kg body weight on a single occasion. Doxycycline hyclate was dissolved in distilled water to obtain a concentration of 10 mg doxycycline per mL. One mL solution was injected into the right brachial vein or given directly into the crop for each kg of body weight. In Groups 1 and 2, food was withheld for 12 h before dosing until 6 h after drug administration. In Group 3, the birds were not fasted prior to treatment, and were allowed access to food throughout the experiment.

From chickens of Group 1, blood samples were drawn via a cannula from the left brachial vein into syringes at 0 (pre-dose), 0.08, 0.17, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h after dosing. In cases of Groups 2 and 3, the sampling times were as follows: 0, 0.17, 0.33, 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h. Plasma samples were separated after centrifugation at 5 °C and 2000 *g* for 10 min, and were stored frozen at –24 °C until analysed.

Sample analysis

Plasma doxycycline concentrations were determined by reversed phase high-performance liquid chromatography (HPLC) with UV detection. Briefly,

the HPLC system was composed of a Hewlett Packard 1090 liquid chromatograph (Hewlett Packard, Waldbron, Germany) and a reversed phase column (Lichrosorb RP 18, 5 µm, 250 × 4 mm, Hewlett Packard). The mobile phase consisted of acetonitrile:methanol:0.02 M oxalic acid:0.02 M Na_2EDTA (20:15:64:1 [v/v]). The flow rate was 1.0 mL/min and the UV detection took place at 345 nm. The determination was performed in two phases: dilution with oxalic acid followed by solid-phase extraction with Sep-Pak C18 cartridge and then reversed phase HPLC measurement. The assay procedure was as follows: 1 mL of plasma was diluted with 10 mL of 0.2 M oxalic acid. After centrifugation (2500 *g*, 10 min) the separated supernatant was passed through a Sep-Pak C18 cartridge. The column was washed with 2 mL of bidistilled water and 1 mL of 10/90 v/v methanol/0.02 M oxalic acid and the investigated compound was eluted with 4 mL of methanol. The eluent was evaporated under a nitrogen stream at 50 °C and the residue was dissolved in 0.5 mL of the mobile phase. Plasma concentrations of doxycycline were quantified against calibration curves of plasma samples spiked with doxycycline reference standard (Sigma, St. Louis, USA).

Linear calibration curves ($r^2 > 0.99$) were obtained in plasma between 0.02–5.0 µg/mL. The limit of the detection was 0.010 µg/mL, while the quantification limit was 0.020 µg/mL. The recovery rates for three different concentrations (0.10, 0.50 and 2.00 µg/mL) were greater than 80% and the intra- and inter-assay coefficients of variation at the same plasma concentrations were less than 10%. The method used was selective for the compound analysed; endogenous interference was not observed on chromatograms.

Pharmacokinetic and statistical analysis of data

The plasma concentration-time data for each individual bird were analysed by one-compartment and two-compartment open models using the MedUsa (Version 1.6) computer program (Várkonyi, 1983). The absolute bioavailability (F_{abs}) was calculated as the ratio of the individual AUC (Area Under the Concentration-Time Curve) values after oral administration to the mean AUC value of the i.v. application. The relative bioavailability (F_{rel}) was obtained as the ratio of the individual AUC after oral administration to nonfasted chickens to the mean AUC after oral application to fasted birds.

Differences in pharmacokinetic data obtained from fasted and nonfasted animals were analysed for statistical significance by the paired Student's *t*-test. Differences of $P < 0.05$ were considered significant.

Results

The mean doxycycline plasma concentration-time profiles after a single i.v. dose and a single oral administration to fasted and nonfasted chickens are presented in Figs 1 and 2. As the curves show the plasma concentrations of doxycycline decreased in a bioexponential manner after both administration routes. A good fit of the observed data to a two-compartment open model was obtained. The values of the calculated pharmacokinetic parameters are summ a-rised in Table 1.

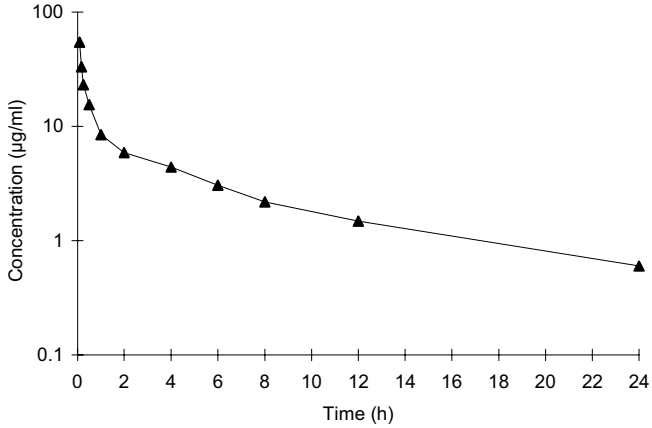

Fig. 1. Mean plasma concentrations of doxycycline after intravenous administration to broiler chickens

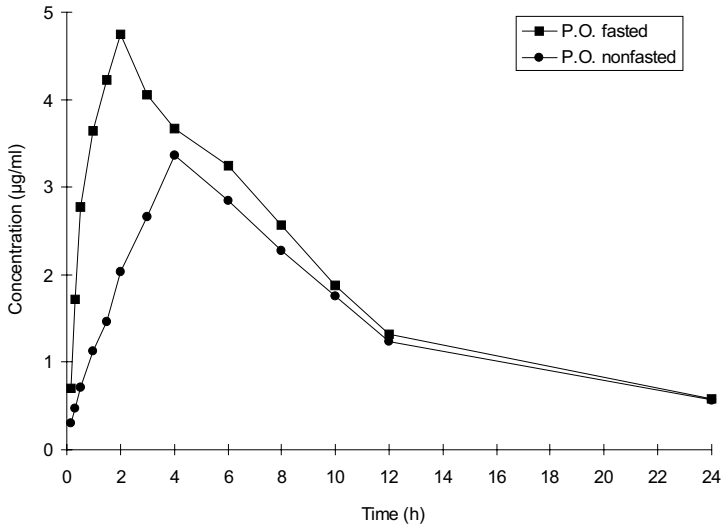

Fig. 2. Mean plasma concentrations of doxycycline after oral administration to fasted and non-fasted broiler chickens

Table 1

Pharmacokinetic parameters of doxycycline following intravenous dosing and oral administration to fasted and nonfasted broiler chickens

Parameter	Administration		
	Intravenous	Oral	
		Fasted	Nonfasted
α (h^{-1})	3.37 ± 0.18	0.21 ± 0.02*	0.43 ± 0.09*
β (h^{-1})	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
K_a (h^{-1})	–	1.85 ± 0.16*	0.62 ± 0.07*
$t_{½a}$ (h)	–	0.39 ± 0.03*	1.23 ± 0.21*
$t_{½\alpha}$ (h)	0.21 ± 0.01	3.61 ± 0.43*	2.18 ± 0.35*
$t_{½\beta}$ (h)	6.78 ± 0.06	8.21 ± 0.37	9.37 ± 0.86
$V_{d\ (area)}$ (L/kg)	1.36 ± 0.06	–	–
Cl_B (L/h·kg)	0.139 ± 0.007	–	–
C_o (µg/mL)	56.63 ± 2.94	–	–
C_{max} (µg/ml)	–	4.47 ± 0.16*	3.07 ± 0.23*
t_{max} (h)	–	1.73 ± 0.06*	3.34 ± 0.21*
AUC (µg·h/mL)	72.68 ± 3.37	53.33 ± 1.80*	44.39 ± 3.19*
MRT (h)	7.97 ± 0.12	11.40 ± 0.38*	13.93 ± 0.67*
F_{abs} (%)	–	73.38 ± 2.47*	61.07 ± 4.39*
F_{rel} (%)	–	–	83.23 ± 5.99

Each value is the mean ± SEM for six chickens; *The marked values are significantly different ($P < 0.05$); α, β = elimination rate constants; K_a = first-order absorption rate constant; $t_{1/2a}$ = absorption half-life; $t_{1/2\alpha}$, $t_{1/2\beta}$ = distribution and elimination half-lives; C_o = initial plasma concentration; C_{max} = maximal plasma concentration; t_{max} = time to reach C_{max}; $V_{d\ (area)}$ = apparent volume of distribution; Cl_B = total body clearance; AUC = area under the curve; MRT = mean residence time; F_{abs} = absolute bioavailability; F_{rel} = relative bioavailability

After i.v. administration, doxycycline was rapidly distributed in the body with a distribution half-life ($t_{1/2\alpha}$) of 0.21 ± 0.01 h. The elimination half-life ($t_{1/2\beta}$) of 6.78 ± 0.06 h was relatively long and resulted from both a low total body clearance (Cl_B) of 0.139 ± 0.007 L/h·kg and large volume of distribution ($V_{d(area)}$) of 1.36 ± 0.06 L/kg.

Following oral administration to fasted chickens, doxycycline was quickly and extensively absorbed as shown by a mean absorption half-life ($t_{1/2a}$) of 0.39 ± 0.03 h, a maximal plasma concentration (C_{max}) of 4.47 ± 0.16 µg/mL and the time to reach the C_{max} (t_{max}) of 1.73 ± 0.06 h. The distribution and the final elimination of the drug from the blood were slower after oral administration than following i.v. dosing. The values for $t_{1/2\alpha}$ and $t_{1/2\beta}$ were 3.61 ± 0.43 h and 8.21 ± 0.37 h, respectively. Bioavailability (F_{abs}) of doxycycline after oral administration to fasted chickens was 73.38 ± 2.47%.

After oral administration to nonfasted birds, the absorption of doxycycline was slower ($t_{1/2a}$ = 1.23 ± 0.21 h, t_{max} = 3.34 ± 0.21 h) and less extensive (C_{max} =

3.07 ± 0.23 μg/mL). These values were significantly ($P < 0.05$) different from those of fasted animals. Mainly due to the lower absorption rate, the calculated value for area under the concentration-time curve (AUC = 44.39 ± 3.19 μg·h/mL) was also significantly different from the AUC of 55.33 ± 1.80 μg·h/mL of fasted birds. The absolute bioavailability in nonfasted chickens was 61.07 ± 4.39%, whereas the relative bioavailability (F_{rel}) was calculated as 83.23 ± 5.99%.

Discussion

Doxycycline has been widely used in chickens to treat and prevent a var i-ety of bacterial infections. Data on the pharmacokinetics of the drug in this sp e-cies are, however, scarce and there is no information on the possible effect of food on its pharmacokinetic profile.

The results obtained from the present study indicate that doxycycline is rapidly and extensively distributed and relatively slowly eliminated after i.v. a d-ministration. Our findings partly differ from those reported by Anadón et al. (1994) who found a similar distribution half-life ($t_{1/2\alpha}$ = 0.23 h) but a faster elimination half-life ($t_{1/2\beta}$ = 4.75 h), and a much lower volume of distribution ($V_{d(area)}$ = 0.28 L/kg) and total body clearance (Cl_B = 0.04 L/h·kg), respectively. Our data are, however, in good agreement with those found by Santos et al. (1996) in 6-week-old turkeys ($t_{1/2\beta}$ = 7.90 h; $V_{d(area)}$ = 1.26 L/kg; Cl_B = 0.11 L/h·kg) or by Dorrestein et al. (1991) in pigeons ($t_{1/2\beta}$ = 6.3 h). Similar results were also reported by Riond et al. (1990) for dogs ($t_{1/2\beta}$ = 6.99 h; $V_{d(area)}$ = 1.01 L/kg; Cl_B = 0.10 L/h·kg).

After oral administration of doxycycline to fasted chickens, the drug was found to be quite rapidly and extensively absorbed from the gastrointestinal tract, followed by a slower distribution and final elimination from the blood than after i.v. dosing. The C_{max}, t_{max} and $t_{1/2\beta}$ values found in our study differed markedly from those (C_{max}: 54.58 μg/mL; t_{max}: 0.35 h; $t_{1/2\beta}$: 6.03 h) reported by Anadón et al. (1994), who used, however, a dose of 20 mg/kg in their study. In 6-week-old fasted turkeys the C_{max} and t_{max} were reported to be 7.40 μg/mL and 2.80 h after a single oral dose of 20 mg/kg (Santos et al., 1996) or 2.22 μg/mL and 1.80 h fo1-lowing a single oral administration of 15 mg/kg (Küng and Wanner, 1994). The absolute bioavailability of 73.4% calculated in our experiment was higher than those found by Anadón et al. (1994) in chickens (41.3%) or by Santos et al. (1996) and by Küng and Wanner (1994) in 6-week-old turkeys (37.0% and 44.6%, respectively).

The presence of food in the intestinal tract significantly decreased and e x-tended the absorption of the drug in chickens, resulting in a lower oral bioavai l-ability (61.1%). The relative bioavailability (fed/fasted) was 83.2%. Thus, the n u-tritional status was seen to influence the bioavailability of doxycycline in chickens as well. The effect was, however, less pronounced than in turkeys (Santos et al.,

1996), where the relative bioavailability varied between 40–60%, except for the 3-week-old birds which showed a relative bioavailability value of 83.7%.

In conclusion, the results obtained in the present study confirm that dox y-cycline has useful pharmacokinetic properties for oral use in chickens. The pre s-ence of food in the intestinal tract appears to have less influence on the bioavai l-ability of the drug in this species than in turkeys.

References

Anadón, A., Martinez-Larranaga, M. R., Diaz, M. J., Bringas, P., Fernandez, M. C., Fernandez-Cruz, M. L., Iturbe, J. and Martinez, M. A. (1994): Pharmacokinetics of doxycycline in broiler chickens. Avian Pathol. **23,** 79–90.

Aronson, A. L. (1980): Pharmacotherapeutics of the newer tetracyclines. JAVMA **176,** 1061–1068.

Dorrestein, G. M., Bruijne, J. J. D. and Vulto, A. (1991): Bioavailability of doxycycline injectables in pigeons. Acta Vet. Scand. **87** (Suppl.) 291–292.

Küng, K. and Wanner, M. (1994): Pharmakokinetik von Doxycycline bei Puten und Vergleich der Futter und Trinkwassermedikation. Arch. Geflügelk. **58,** 84–88.

Meijer, L. A., Ceyssens, K. G. F., de Greve, B. I. J. A. C. and de Bruijn, W. (1993): Pharmacokinetics and bioavailability of doxycycline hyclate after oral administration in calves. Vet. Quarterly **15,** 1–5.

Riond, I.-L., Vaden, S. L. and Riviere, J. E. (1990): Comparative pharmacokinetics of doxycycline in cats and dogs. J. Vet. Pharmacol. Therap. **13,** 415–424.

Santos, M. D. F., Vermeersch, H., Remon, J. P., Schelkens, M., De Backer, P., Van Bree, H. I. I., Ducatelle, R. and Haesebrouck, F. (1996): Pharmacokinetics and bioavailability of doxycycline in turkeys. J. Vet. Pharmacol. Therap. **19,** 274–280.

Santos, M. D. F., Vermeersch, H., Remon, J. P., Schelkens, M., De Backer, P., Ducatelle, R. and Haesebrouck, F. (1997): Administration of doxycycline hydrochloride via drinking water to turkeys under laboratory and field conditions. Poultry Sci. **76,** 1342–1348.

Shaw, D. H. and Rubin, S. I. (1986): Pharmacologic activity of doxycycline. JAVMA **189,** 808–810.

Várkonyi, P. (1983): A calculator package for pharmacokinetic application. Comp. Prog. Biomed. **17,** 277–282.

Welling, P. G., Koch, P. A. and Curtis, C. L. (1977): Bioavailability of tetracycline and doxycycline in fasted and nonfasted subjects. Antimicrob. Agents Chemother. **11,** 462–469.