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Comparative Bio-equivalence Study of Dolistin® and Colidox® in Chickens

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Abstract: The comparative bio-equivalence of Dolistin® and Colidox® at a dose rate of 10 mg doxycycline/kg b. wt. was studied in 48 clinically normal broiler chickens. After oral administration, plasma levels of doxycyline peaked after 2 hours post-dosing without significant differences between the two products and it could be detected therapeutically and exceeded the minimum inhibitory concentration (MIC) for most micro-organisms sensitive to doxycycline for 12 hours. The disposition kinetics of doxycycline in the two products following oral administration revealed that the maximum plasma concentration ($C_{max.}$) were 22.65 and 21.80 µg/ml and attained at ($t_{max.}$) 2.10 and 2.20 hours, respectively. Doxycycline in both of the products was eliminated with half-lives ($t_{0.5â}$) equal to 7.70 and 6.93 hours, respectively. The mean systemic bioavailabilities of doxycycline in both of the products after oral administration in chickens were 80.60 and 79.70%, respectively. Doxycycline residues in Dolistin® and Colidox® after repeated oral administration in chickens were detected in all the tested tissues (liver, kidney, lung and muscle). Doxycycline of both of the products was completely cleared from the chicken's bodies 6 days after the last oral dose. Colistin in both of the products could not be detected in blood or tissues after oral administration that makes it the antibiotic of choice for the treatment of enteric infections caused by organisms sensitive to colistin. It was concluded that doxycycline in the form of Dolistin® and Colidox® needs 6 days for withdrawal in chickens and a dose equivalent to 20 mg doxycycline/kg a day is better to keep the plasma concentration higher than the MIC.

Key words: Tetracyclines · doxycycline · bioavailability · chickens

INTRODUCTION

Tetracyclines are of great clinical importance because they posses a wide range of antimicrobial activity against aerobic and anaerobic Gram-positive and Gram negative bacteria [1]. Also they are effective against some microorganisms that are resistant to cell-wall-inhibitor antimicrobial agents such as *Rickettsia, Mycoplasma pneumoniae*, *Chalamydia.*, *Ureplasma spp* and some atypical *Mycobacteria* and *Plasmodium* spp. [2].

Doxycycline is a second generation tetracycline mainly active against Gram-positive and Gram-negative aerobic and anaerobic bacteria as well as *Mycoplasma*, *Chlamydia, Rickettsia* and *Spirochetes*. It has a low affinity for calcium and is relatively more stable in aqueous solution [3].

Doxycycline has a broad spectrum action against a wide range of Gram-positive and Gram-negative pathogenic bacteria including some penicillin resistant strains. The organisms that are sensitive to tetracycline in concentration usually achieved in the body during treatment include, *Bacillus anthracis, Bordetella sp., Straphylococci, Streptocolcci, Mycoplasma,* certain *Rickettsia*, *Chlamydia*, *Actinomyces* spp., as well as large viruses are also sensitive to doxycycline [4]. The exact site involved in its antimicrobial activity has not been clarified, but all tetracyclines antibiotics bind reversibly to the bacterial 30 S ribosomal unit and inhibit the protein synthesis, perhaps by several mechanisms [5]. Doxycycline is readily absorbed after oral administration from gastrointestinal tract and absorption is not significantly affected by the presence of food in the gastrointestinal tract [6].

Colistin (polymyxin E) is an antibiotic produced by *Bacillus polymyxa* subsp. *colistinus* [7]. It consists of a cyclic heptapeptide and a side-chain of three amino acids achylated at the N-terminus by a fatty acid. It is a complex mixture of at least 30 different components [8]. The two main components are colistin A (polymyxin E1) and colistin B (polymyxin E2) which are only different in the

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fatty acid side chain. Colistin is acting mainly against Gram-negative bacteria. As it is not absorbed from the gastro-intestinal tract, it develops its main action in the intestine against different *Coli* and *Salmonella* strains and *Enterobacter*. A further advantage of colistin is the fact that bacterial endotoxins (*E.coli*) are inactivated [9].

The present work was planned to study the comparative blood concentrations, bioavailability and residues of both preparations to determine their usefulness against bacterial infections affecting poultry.

MATERIALS AND METHODS

Drugs

A) Dolistin® (MEDMAC. CO. Jordan) water soluble powder:

Each 100 g contains:

Doxycycline (HCL)	20 g
Colistin base (as sulphate)	60,000,000 IU

B) Colidox® (BREMER pharma GMBH, Germany) water soluble power.

Each 100 g contains:

Doxycycline (HCL)	10 g
Colistin base (as sulphate)	120,000,000 IU

Chickens and experimental design: Forty eight clinically normal Hubbard chickens of 2-3 months, weighing about 1800-2200 g, were randomly chosen from Tanta Poultry Farm, Egypt. Chickens were fed on a balanced ration free from antibiotic for 2 weeks to withdraw any antibiotic residues. Before administration, chickens were grouped into 4 groups, group 1 and 2 were 6 chickens each for studying the pharmacokinetic parameters and the bioavailability and groups 3 and 4 were 18 chickens each for studying the tissue residue.

Pharmacokinetic parameters and the bioavailability of Dolistin® amd Colidox® following oral administration: The pharmacokinetic parameters and the bioabailability of Dolistin® and Colidox® were studied in six normal chickens for each product. Chickens were injected intravenously into the left wing vein with 10 mg pure doxcycycline/kg b. wt. These chickens were left for two weeks to insure complete secretion of the tested antibiotics from their bodies. Then the tested chickens were given orally Dolistin® (Group 1) or Colidox ®(Group 2) in a dose equivalent to 10 mg doxycycline base/kg b. wt.

Tissue residues of Dolistin® and Colidox® following repeated oral administration: Tissue residues of Dolistin® (group 3) and Colidox® (group 4) were determined in 36 normal chickens following repeated oral administration of 10 mg doxycycline base/kg b. wt. from both drugs once daily for five consecutive days. After the end of the fifth day of repeated oral administration, three chickens were euthanized at 24, 48, 72, 96, 120 and 144 hours for both preparations.

Samples: A. *Plasma samples*: About one milliliter of blood was taken in a heparinized tube from the right wing vein, after administration of each drug. Blood samples were collected after 5, 10, 15, 30 minutes, 1, 2, 4, 6, 8, 12 and 24 hours after the single intravenous and oral administration (Groups 1 and 2). The blood samples were centrifuged at 3000 rpm for 5 minutes and the plasma was separated.

Tissue samples: After the end of the fifth day of repeated oral administration of Dolistin® and Colidox®, three chickens were slaughtered at 24, 48, 72, 96, 120 and 144 hours. From each slaughtered chicken, plasma, lung, liver, kidney and muscle samples were taken for drug assay. Samples were frozen and stored at -20 °C until assayed for both doxycycline and colistin.

Analytical procedures: A. *Doxycycline HCl in plasma and tissues*: detection of doxycycline in plasma and tissue samples was done according to Alsarra *et al*. [10]. To a 400 µl plasma or 400 mg crushed tissue sample previously spiked with the 200 µl (2.0µg/ml) of internal standard (tetracycline HCl) add 300 µl of trichloroacetic acid solution (10% in water). The mixture was vortex mixed for 30 seconds and then centrifuged for 10 min at 13000 r.p.m. and 20 µl aliquot samples were injected to the HPLC system. The running conditions were carried out by using a Waters (Bedford, MA, USA) HPLC system, a Bondapack C18 column (5 µm, 3.9 mm I.D. x 150 mm), a guard column of the same material was used, a Schmadzu (Shimadzu Corporation, Kyoto, Japan) UV detector with the wavelength was set on 346 nm. The mobile phase consisted of 72% water, 28% acetonitrile, (v/v). At final, the solution contained 0.02 M oxalic acid and 0.0005 M EDTA, (pH 2.15). The mobile phase was prepared fresh, degassed and filtered daily by passing it through a 0.22 µm membrane filter (Millipore, Bedford, MA, USA). The mobile phase was pumped isocratically at a flow rate of 0.80 ml/min at the room temperature.

Colistin sulphate: Detection of colistin was done according to Li *et al*. [11]. In a 1.5-ml polypropylene tube, mix 250 µl plasma sample with 20 µl of the internal

standard (5mg netilmicin sulfate in water). To the previous mixture 50 µl methanol-10% tichloroacetic acid (50/50, v/v) was added and the mixture was vortex mixed for 1 min and centrifuged at 1000 *x g* for 10 min at 4°C. The supernatant was transferred to another 1.5-ml polypropylene tube and mixed with 10 µl of 1 *N* sodium hydroxide. After being vortex mixed for 1 min, 250 µl of methanol–0.01 *N* hydrochloric acid (50:50, v/v) was added followed by vortex mixing for 1 min. The final solution was taken to the conditioned solid phase extraction column. The C_{18} SPE (JT Baker) was conditioned with 1 ml methanol followed by 1 ml carbonate buffer (1% w/w, pH 10) then the previously treated plasma sample was passed through the SPE column. The extraction cartridge was washed with 1 ml carbonate buffer and the drugs were left for 10 min to be drivatized after adding 30 µl of the drivatizing agent 9-fluorenylmethyl chloroformate (100 m*M* in acetonitrile). After drying the derivatives were eluted with 900 µl acetone, and the eluate mixed with 600 µl boric acid solution (0.2 *M*). After vortex-mixing for 2 min, 20 µl was injected into the HPLC system. The running conditions were carried out by using a Waters (Bedford, MA, USA) HPLC system, a Beckman Ultrasphere C_{18} column (5 µm, 250 x 4.6 ID). The mobile phase of acetonitrile –tetrahydrofuran–water (87:4:13, v/v) was degassed by sonication prior to use and pumped at a flow rate of 1 ml/min. The detection was done by using a Waters 470 scanning fluorescence detector at an excitation wavelength of 260 nm and an emission wavelength of 315 nm.

Pharmacokinetic analysis: The concentration- time curve of the two tested antibiotics followed a two-compartment open model. The pharmacokinetic parameters were calculated according to Ritschel]12] and Baggot [13, 14].

RESULTS

Following a single intravenous injection of 10 mg doxcycyline base/kg b. wt. in chickens (groups 1 and 2), it could be detected in blood till 24 hours post- injection and followed a two-compartment open model (Table 1 and Fig 1a and b). Plasma levels of doxcycyline and colistin after oral administration in chickens of groups (1 and 2) are recorded in Table 2 and shown in Fig. 2 a and b that showed the maximum doxcycyline concentration is around 2 hours post-administration without significant differences between the two groups. The pharmacokinetic parameters of doxcycyline in Dolistin® and Colidox® following intravenous and oral administration of 10 mg

Table 1: Plasma levels of a doxcycyline in Dolistin® and Colidox® groups in normal chickens following intravenous injections of 10 mg doxcycyline base/kg b. wt. (n=6)

Time after administration (h)	G1 Dolistin®	G2 Colidox®
-5min	40.60±5.00	41.80±5.35
0.25	35.40±4.00	36.45±5.30
0.5	33.45±5.80	33.30±3.90
1	28.30±3.20	29.80±0.35
2	18.55±2.80	20.30±2.35
4	7.80±1.40	10.10±1.75
6	5.20±0.60	6.90±1.60
8	4.90±0.95	4.50±0.80
12	3.20±0.80	2.30±0.85
24	0.95±0.35	0.80±0.25

Table 2: Plasma levels of Dolistin® and Colidox® in normal chickens following oral administration of 10 mg doxcycyline/Kg b. wt. (n = 6)

Time after	Dolistin®		Colidox®	
administration	Doxcycyline	Colistin	Doxcycyline	Colistine
-5min	0.50±0.60	ND	0.75±0.15	ND
0.25	3.10±0.58	ND	3.00±0.75	ND
0.50	7.90±1.00	ND	8.20±1.40	ND
1	15.30±2.90	ND	17.00±3.00	ND
2	21.00±3.25	ND	22.10±3.60	ND
4	16.50±2.70	ND	16.30±2.80	ND
6	12.20±1.90	ND	12.00±1.00	ND
8	10.00±2.10	ND	10.10±1.65	ND
12	5.75±1.30	ND	5.60±0.75	ND
24	1.60±0.67	ND	1.40±0.80	ND

ND = Not detected

Table 3: Pharmacokinetic parameters of doxycycline in Dolistin® and Colidox® after oral administration of 10 mg doxycycline/kg b. wt. in normal chickens

Parameter	Unit	Group 1 Dolistin®	Group 2 Colidox®
A	µg/ml	30.60±3.55	30.90±3.70
K_{ab}	h^{-1}	1.20±0.09	1.18±0.08
$t_{0.5(ab)}$	h	0.58±0.09	0.58±0.07
C_{max}	µg/ml	22.65±3.10	21.80±2.60
T_{max}	h	2.10±0.70	2.20±0.85
B	µg/ml	28.90±3.20	27.80±2.90
K_{el}	h^{-1}	0.09±0.008	0.10±0.009
$t_{0.5(â)}$	h	7.70±1.40	6.93±1.35
AUC	µg/ml/h	18.50±3.20	17.95±3.40
F	%	80.60±7.40	79.70±8.20

Pharmacokinetic abbreviations: A&B: Zero-time serum concentration intercepts of basic oral disposition curve (µg/ml).K_{ab}: Apparent first order absorption rate constant (h^{-1}). $t_{0.5(ab)}$: The absorption half time (h). C_{max}: Maximum serum concentration of drug after extravascular administration (µg/ml). T_{max}: The time at which the maximum serum concentration achieved after extravascular administration (h). K_{el}: Firs-order transfer rate constant for the disappearance of the drug from the central compartment (h^{-1}). $t_{0.5(â)}$: Elimination half life (h). AUC: Total area under the serum drug concentration versus time curve from t=0 to t = 8 (µg/ml/h). F: bioavailability

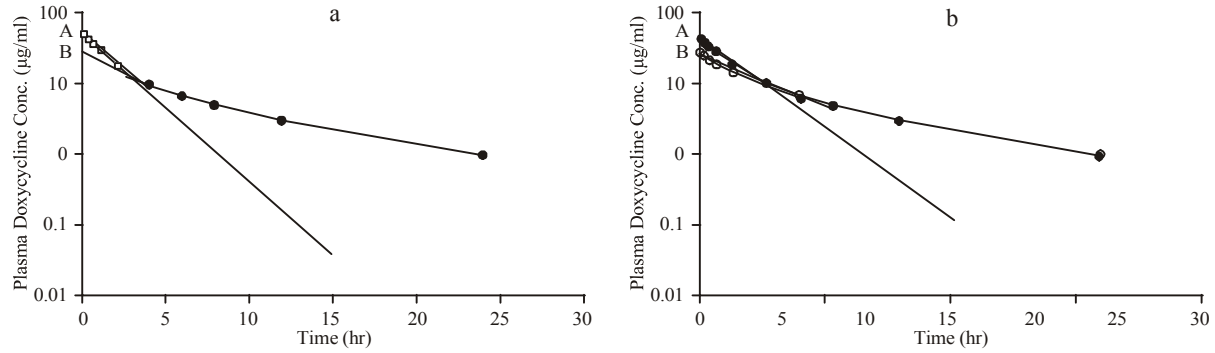

Fig. 1: Semilog graph depicting the time plasma concentration of doxycycline in broiler chickens after intravenous administration of doxycycline base in Dolistin® (a) and Colidox® (b)

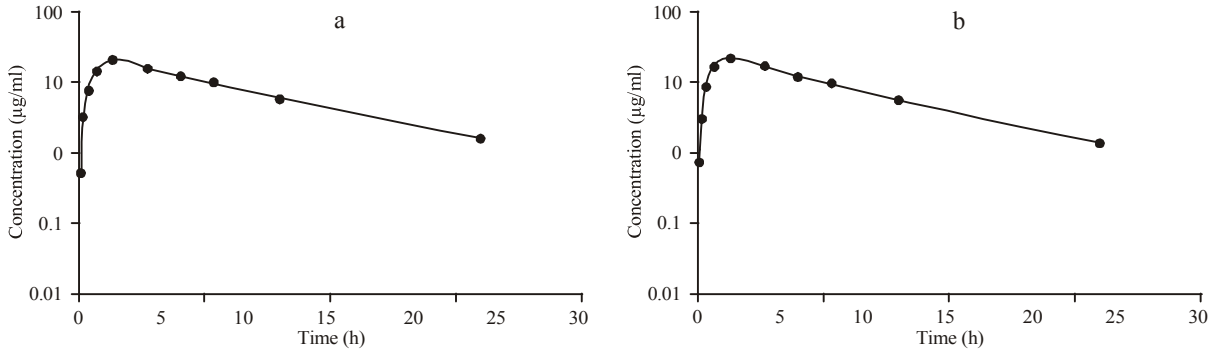

Fig. 2: Semilog graph depicting the time plasma concentration of doxycycline in broiler chickens after oral administration of Dolistin® (a) and Colidox® (b) in normal broiler chickens at doses equivalent to 10 mg doxycycline /kg b. wt. in normal chickens

Table 4: Plasma (µg/ml) and tissue (µg/g) concentrations of doxcycyline in Dolistin® in chickens following repeated oral administrations of 10 mg doxcycyline base/ kg.b.wt. once daily for five consecutive days (n=3)

	Time after the last dose (h)					
Plasma and tissues	24 hours	48 hours	72 hours	96 hours	120 hours	144 hours
Plasma	2.60	0.80	0.00	0.00	0.00	0.00
Lung	39.80	17.00	9.30	1.80	0.00	0.00
Liver	44.50	27.80	11.30	2.60	0.45	0.00
Kidney	45.30	30.15	13.35	4.00	1.05	0.00
Muscles	17.50	8.00	2.10	0.30	0.00	0.00

Table 5: Plasma (µg/ml) and tissue (µg/g) concentrations of doxcycyline in Colidox® in chickens following repeated oral administrations of 10 mg doxcycyline base/kg b. wt. once daily for five consecutive days (n=3)

	Time after the last dose (h)					
Plasma and tissues	24 hours	48 hours	72 hours	96 hours	120 hours	144 hours
Plasma	3.00	1.20	0.35	0.0010.3	0.00	0.00
Lung	35.00	20.00	10.30	2.20	0.70	0.00
Liver	41.80	26.00	10.60	1.95	0.00	0.00
Kidney	47.00	35.80	16.75	5.60	1.00	0.00
Muscles	15.30	6.90	1.80	0.00	0.00	0.00

doxcycyline base/kg b. wt. are recorded (Table 3). The plasma and tissue residue concentrations of doxycycline after dosing the chickens with the two preparations at a dose rate 10 mg doxycycline/kg b. wt daily for 5 consecutive days are recoded in Table 4 and 5.

DISCUSSION

The plasma concentrations of doxycycline 24 hours after oral dosing of both of the products at a dose rate 10 mg doxycycline/kg b. wt. were 1.6 and 1.40 µg/ml respectively. These values were lower than the MIC of doxycycline which is 4µg/ml according to [4]. In other studies, doxcycyline has a shorter [$t_{1/2(â)}$] in chickens than other domestic species, however, metabolism of the drug did not seem to be the cause of the differences in drug elimination among species. Moreover, photodiode array detection of an HPLC column effluent and mass-spectrometric analysis of serum and urine of calves, pigs, cats, dogs and man confirmed that doxcycyline was metabolized [15].

The disposition kinetic parameters of doxcycyline in Dolistin® and Colidox® following oral administration of 10 mg doxcycyline base/kg b. wt. revealed that the maximum blood concentration C_{max} were 22.65 and 21.80 µg/ml and attained at t_{max} 2.10 and 2.20 hours, respectively. The area under the curve of serum doxcycyline in Dolistin® and Colidox® concentrations-time curve following oral and intravenous administration in the same chickens produced a mean systemic bioavailability equal to 80.60 and 79.70%, respectively. These values indicate a good absorption of doxcycyline in both Dolistin® and Colidox® from the GIT, since the drugs could be detected at earlier sample collection, 5 minutes, with values of 0.50 and 0.75µg/ml, respectively. In other studies; after oral administration of 20 mg doxcycyline/kg, it was rapidly absorbed and the average systemic bioavailability in mice and humans was 90-95% [16]. In addition, the mean maximum plasma concentration in chicken as mentioned above is different than that recorded by Shaw and Rubin [16] who recorded it at 54.58 µg/ml in broilers and 5.3 µg/ml in laying hens after a single oral administration of 20 mg/kg b. wt. An approximate target concentration could be between 0.5 and 4 µg/ml. moreover, plasma doxcycyline concentrations at 24 hours for 10 mg/kg b. wt. dosage of the two preparations were 1.60 and 1.40 µg/ml respectively which is lower than the MIC. On these bases, the plasma doxycycyline concentration achieved after oral administration of 20 mg/kg b. wt./day should be appropriate for control of avian diseases.

For oral administration of 10 mg doxycycline/kg b. wt. of both of the products, for five successive days, the withdrawal time of 6 days might be adequate to predict that the concentration in edible tissues is below the accepted tolerance levels of 0.600 µg/g kidney, 0.300 µg/g liver and 0.10 µg/g muscle [17]. From the public health viewpoint and according to the Food Market Institute report [18] it is important to consider that recommendation in the light of an overall risk-benefit assessment for consumers of these food-producing animals. Plasma level and tissue residues of doxycycline of Dolistin® and Colidox® in euthanized chickens following repeated oral administration of a dose rate 10 mg doxycycline base/kg b. wt. of the two preparations once daily for 5 consecutive days represented a wide spread distribution of doxycycline in both Dolistin® and Colidox® in the lung, liver, kidney and muscle. The liver, kidney and lung contained the highest drug residues while the lowest drug residue was in the plasma. Doxycycline of Dolistin® and Colidox® was completely cleared from the plasma and all tissues on day 6 (144 hrs) after the last dose. Several studies have shown that doxycycline is readily available for tissue distribution [17, 19, 20]. Although doxycyline is a highly lipid soluble tetracycline derivatives [15] the data found that doxycyline levels in the kidney, liver, lung and muscle were lower than the concurrent levels in plasma 12 hours after dosing. These values were lower than that reported in cattle by Gallo and Berg [6] who found a higher level of doxycycline in the muscle than that in the serum 12 hours after the end of dosing. These results indicated that the rate of distribution of doxycycline in chickens is lower than that in other species.

Colistin could not be determined in all plasma or tissue samples after the last dose of repeated oral administration of both of the preparations that indicated that colistin is not absorbable after oral administration which is consistent with the results of Brander *et al*. [9] and Tomast *et al*. [21].

It could be concluded that doxycycline in Dolistin® and Colidox® level reached the peak in plasma after 2 hours from the single oral administration and could be detected in a level exceeds the MIC over 12 hours and that after 24 hours is lower than the MIC that advices to make the daily oral dose of 20 mg doxycycline/kg b. wt is appropriate. High bioavailability of doxycycline in Dolistin® (80.60 %) after oral administration indicated a good absorption from the GIT. The withdrawal time of doxycycline of both Dolistin® and Colidox® is 6 days after oral administration. Colistin is not absorbed from the GIT after oral route of administration of both of the products. That factor indicates that colistin is only suitable for enteric G-ve organisms.

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