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Research paper

Bio-equivalence study of two oral doxycycline formulations (doxycycline kela 75%® and mebcodox 75%®) in broiler Chickens

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Abstract

The present study was designed to assess the comparative bio-equivalence of Doxycycline Kela 75%® and Mebcodox 75%® in healthy broiler chickens after oral administration of both products in a dose of 20 mg doxycycline base/kg.b.wt. Twenty four broiler chickens were divided into two groups. The first group was designed to study the pharmacokinetics of Doxycycline Kela 75%®, while the 2nd group was designed to study the pharmacokinetics of Mebcodox 75%®. Each broiler chickens in both groups were orally administered with 20 mg doxycycline base/kg.b.wt. Blood samples were obtained from the wing vein and collected immediately before and at 0.08, 0.16, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after a single oral administration The disposition kinetics of Doxycycline Kela 75%® and Mebcodox 75%® following oral administration of 20 mg doxycycline base/kg.b.wt. revealed that the maximum blood concentration [C_{max}] were 3.35 and 3.28 µg/ml and attained at [t_{max}] of 0.97 and 0.99 hours, respectively.
In conclusion: Mebcodox 75%® is bioequivalent to Doxycycline Kela 75%® since the ratios of C_{max}, AUC_{0-24} and $AUC_{0-\infty}$ (T/R) was 0.97, 0.95 and 0.94 respectively. These are within the bioequivalence acceptance range. Mebcodox 75%® and Doxycycline Kela 75%® are therefore bioequivalent and interchangeable.

Keywords: *Bioequivalence, Chickens; Doxycycline; oral; Pharmacokinetics.*

1. Introduction

Doxycycline is a tetracycline derivative with broad spectrum activity against Gram-positive and Gram-negative aerobic and anaerobic bacteria: Spirochaete, Mycoplasma, Chlamydia and Rickettsia species (Shaw and Rubin, 1986; Dorrestein et al., 1990; Goren et al., 1998; Riviere and Spoo, 2003). Doxycycline has some advantages over older tetracycline derivatives including higher lipid solubility, better bioavailability and tissue distribution, longer elimination half-life, and lower affinity for calcium ions (Aronson, 1980). The pharmacokinetics of doxycyclin after oral administration have been studied in healthy chickens (Anadon et al., 1994; Laczay et al., 2001) and in Mycoplasma gallisepticum-affected broilers (Ismail and El-Kattan, 2004; Perez et al., 2006).
Doxycycline is a semi-synthetic bacteriostatic tetracycline and a broad-spectrum antibiotic against Gram-negative and Gram-positive aerobic and anaerobic bacteria, Rickettsiae, Chlamydiae, Mycoplasmas and some protozoa (Jha et al., 1989; Prats et al., 2005). Pharmacokinetics properties of doxycycline are superior than older tetracycline, in terms of higher lipid solubility, complete absorption, better tissue distribution, longer elimination half-life and lower affinity for calcium (Riond and Riviere, 1990; Goren et al., 1988). The in vitro antimicrobial activity of doxycycline is more effective than other tetracycline for the treatment of respiratory, urinary and gastrointestinal tract diseases (Abd El-Aty et al., 2004).
Doxycycline, a member of tetracycline's, is a structural isomer of the parent molecule and is synthesized from oxytetracycline or methacycline. It differs from tetracycline in that it is more lipophilic (5–10 times) and has a greater plasma protein binding capacity, the overall resulting in higher tissue penetration, larger volume of distribution, better antimicrobial properties and prolonged half-life both in humans and animals. The antibacterial effect of doxycycline is best described as interfering with the binding of aminoacyl-tRNA to the mRNA ribosome complex, thereby hindering the protein synthesis in growing and/or multiplying organisms. Doxycycline is an inexpensive, broad-spectrum antibacterial agent that remains the drug of first choice for several infections such as atypical pneumonias, skin and soft tissue, genitourinary infection including gonorrhea, syphilis, non-specific urethritis, and prostatitis (Cunha et al., 1982).
It has a high relative liposolubility (5- to 10-fold increases in relatio n to older tetracyc1ines) which readily compensates for the high protein binding (Barza et al., 1975) and act against staphylococci, streptococci and anaerobic bacteria (English, 1966; Williamson, 1968; Chow et al., 1975). All these characteristics support the notion that doxycycline may have therapeutic usefulness in veterinary medicine. Since doxycycline was introduced into modem drug therapy by Schach Von Wittenau & Delahunt (1966) many of its pharmacokinetic and pharmacodynamic characteristics have been studied in detail mainly in humans (Fabre et al., 1971; Saux et al., 1981; Cars and Ryan, 1988). Doxycycline has several important advantages over other tetracycline analogues: absorption is almost complete, tissue penetration is good, elimination is slower necessitating only one daily dose, the elimination rate is irrespective of renal function and more than 90%

of the dose is recovered as undegraded drug from urine and faeces. Although few pharmacokinetic studies have been done in birds, doxycycline is often used to treat avian infectious diseases such as colibacillosis, salmonellosis, staphylococcosis, mycoplasmosis and chlamydiosis, (George et al., 1977; Jakoby, 1979; Gylstorff et al., 1984; Goren et al., 1988; Dorrestein et al., 1990).

The bioavailability and bioequivalence studies play an important role in determining therapeutic efficacy to register the generic drug products according to the Food and Drug Administration (FDA) regulations (Chen et al., 2001). Bioavailability is defined as the rate and extent to which an active drug ingredient is absorbed and becomes available at the site of drug action. In case of bioequivalence it is defined as statistically equivalent bioavailability between two products at the same molar dose of the therapeutic moiety under similar experimental conditions (Chen et al., 2001; Toutain and Bousquet-Melou, 2004). The drug products are said to be bioequivalent if they are pharmaceutical equivalents or pharmaceutical alternatives and if their rate and extent of absorption do not show a significant differences statistically according to the FDA regulations (Chen et al., 2001).

The aim of this study is to evaluate bioequivalence of two oral doxycycline formulations (Doxycycline Kela 75%® and Mebcodox 75%®) after oral administration of a single dose in broiler chickens.

2. Materials and methods

2.1. Drugs

Doxycycline Kela 75%®: is manufactured by Kela Veterinaria NV, Belgium, as water soluble powder. Each 1gm contains 750 mg doxycycline base (as doxycycline hyclate) and it was used as a reference product.

Mebcodox 75%®: is manufactured by Boston Company, Mebco Vet Division, Egypt, as water soluble powder. Each 1gm contains 750 mg doxycycline base (as doxycycline hyclate) and it was used as test product.

2.2. Broiler chickens and experimental design

Twenty four healthy broiler chickens (30 days old and weighing 1.6 – 1.8 kg) were obtained from Benha private poultry farm, Egypt. They were kept individually in cages, within a ventilated, heated room (20˚C), and 14 hours of day light. They received a standard commercial ration free from any antibiotics to insure complete clearance of any anti-bacterial substances from their bodies. Water was offered ad-libitum.

2.3. Bioequivalence study

Broiler chickens were used to study the bio-equivalence of Doxycycline Kela 75%® and Mebcodox 75%® after oral administration. Broiler chickens were divided into two groups. The 1st group (12 broiler chickens) was used to study the pharmacokinetics of Doxycycline Kela 75%®. The 2nd group (12 broiler chickens) was used to study the pharmacokinetics of Mebcodox 75%®. Broiler chickens in the 1st group were administered orally (intra-crop) with Doxycycline Kela 75%® in a dose of 20 mg doxycycline base/kg.b.wt, while broiler chickens in the 2nd group were administered orally with Mebcodox 75%® in a dose of 20 mg doxycycline base/kg.b.wt).

2.4. Blood samples

Blood samples were obtained from the wing vein (1 ml) and collected in test tubes immediately before and at 0.08, 0.16, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after a single oral administration (groups 1 and 2). Samples were centrifuged at 3000 rpm for 10 minutes and the obtained sera were used for the estimation of doxycycline concentration. The serum samples were stored at −20˚C until analysis, and the assay was performed within a week of obtainment.

2.5. Analytical procedure

Rapid agar-diffusion assay for the quantitative determination of doxycycline in small volumes of blood by using Bacillus subtilis (ATCC 6633) according to Arret et al., (1971). The organism was washed from the agar slant (which has been incubated for 24 hour 37˚c). The resulting growth was washed with 50 ml of sterile normal physiological saline. The resulting suspension was centrifuged at 3000 r.p.m. for 10 minutes and the supernatant was resuspended in 50-70 ml normal saline and heated for 30 minutes at 70˚c. The final spore suspension was diluted with saline to obtain a density of 10^7 spores/ml by using Mc-forland and nephelometer barium sulphate standard. The diluted suspension was stored in refrigerator at 4˚c till used. About 1 ml of the suspension of Bacillus subtilis 10^7/ml was added to 100 ml agar at 55-60˚c. The mixture was shaken thoroughly till complete mixing of the test organism with agar. Petri dishes (20 cm x 20 cm) were used; about 25 ml of inoculated medium were poured to each dish by using sterile cylinder. After complete solidification, six wells were made on the surface of inoculated agar using stainless steel cylinder. The wells of each plate were filled with the serum sample. The plates were incubated at 37 ˚c for 16-18 hours. The diameter of each inhibition zone was measured.

The calibration curves of serum were prepared with different concentrations between 0.1 and 20 μg/mL using blank chickens serum.

Thereafter, the diameters of inhibition zones were measured with the aid of a transparent rule to the nearest millimeter. Each sample was replicated three times and analyzed similarly. The plot of doxycycline serum concentrations versus diameters of inhibition zone was linear with a correlation coefficient of 0.993. Serum concentrations of doxycycline were determined by comparing the zone of inhibition diameters with the standard curve. The absence of interfering endogenous compounds was demonstrated in antibacterial-free plasma obtained at time 0 (pretreatment) which showed no visible zone of inhibition around the impregnated disks. The limit of quantification (LOQ) defined visually as the smallest amount of drug that still produced a clearly distinguishable inhibition zone around the edges of doxycycline contained pores on nutrient agar media was 0.1 μg/ml.

2.6. Pharmacokinetics analysis

Serum concentrations of doxycycline versus time data obtained during the study were utilized for calculating various pharmacokinetic variables using a compartmental and non-compartmental analysis using computerized program, WinNonline 4.1 (Pharsight, USA).

The peak concentrations, C_{max} and time to peak, T_{max} were obtained from the serum concentration-time data directly. The areas under the serum concentration of doxycycline time curves from time 0 to the last sample collected (AUC_{0-24}) were calculated using linear trapezoidal method (Baggot, 2001). While $AUC_{0-\infty}$ was derived from $AUC_{0-24} + AUC_{24-\infty}$, where $AUC_{24-\infty} = C_{24}/ß$. For bioequivalence evaluation, the ratios of C_{max} (T/R), AUC_{0-24} (T/R) and $AUC_{0-\infty}$ (T/R) were calculated. Values within the bioequivalence acceptable range at 90% confidence interval, 0.80 – 1.25 were considered for accepting the null hypothesis of bioequivalence between the reference and the test brands (EMEA, 2002, 2006).

3. Results

The mean serum concentrations of doxycycline in Doxycycline Kela 75%® and Mebcodox 75%® following oral administration of 20 mg doxycycline base/kg.b.wt. in broiler chickens are shown (Table 1 and Figure 1).

Table 1: Mean (X ± S.E) Serum Concentrations (mg/ml) Of Doxycycline in Doxycycline Kela 75%® and Mebcodox 75%® Following Oral Administration of 20 mg Doxycycline Base/kg. b.wt. in Broiler Chickens (N = 12)

Time post Administration (hour)	Mean serum concentration (µg/ml) Doxycycline Kela 75%® (Reference)	Mebcodox 75%® (Test)
	0.81±0.04	0.75±0.03
0.08	1.37±0.08	1.29±0.05
0.16	2.29±0.14	2.19±0.11
0.25	3.18±0.16	3.11±0.17
0.5	3.69±0.18	3.58±0.17
1	2.71±0.13	2.68±0.15
2	2.19±0.12	2.11±0.14
4	1.93±0.04	1.87±0.06
6	1.38±0.04	1.28±0.05
8	0.91±0.02	0.87±0.03
12	0.41±0.02	0.37±0.02
24		

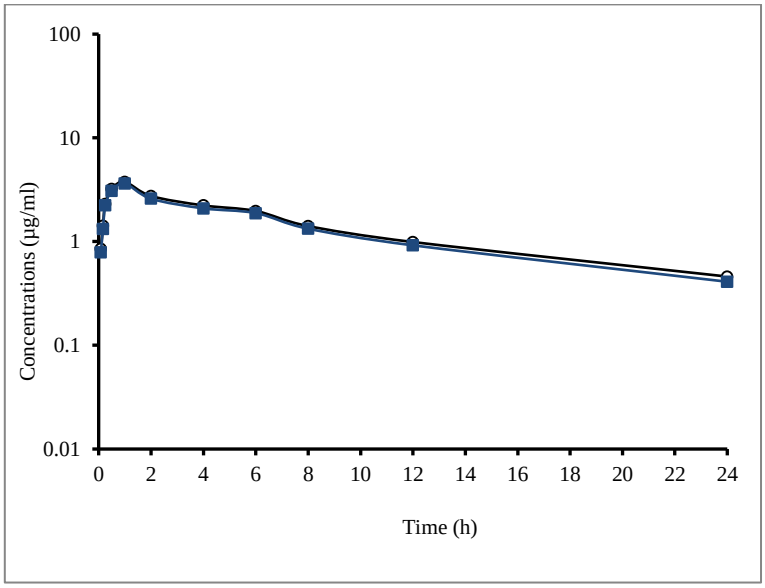

Fig. 1: Semilogarthimic Plot Showing The Serum Concentrations-Time Profile of Doxycycline in Doxycycline Kela 75%® (○) and Mebcodox 75%® (■) Following Oral Administration of 20 mg Doxycycline Base/kg. b.wt. in Broiler Chickens (N = 12).

The mean pharmacokinetic parameters of doxycycline in Doxycycline Kela 75%® and Mebcodox 75%® after oral administration of 20 mg doxycycline base/kg.b.wt. in broiler chickens are shown (Table 2).

Table 2: Mean (X ± S.E) pharmacokinetic parameters of doxycycline in Doxycycline Kela 75%® and Mebcodox 75%® following oral administration of 20 mg doxycycline base/kg.b.wt. in broiler chickens (n = 12)

Parameter	Unit	Doxycycline Kela 75% ® (Reference)	Mebcodox 75%® (Test)
K_{ab}	h^{-1}	3.54 ± 0.25	3.40 ± 0.13
K_{el}	h^{-1}	0.073 ± 0.001	0.076 ± 0.001
$t_{1/2(ab)}$	h	0.195 ± 0.007	0.203 ± 0.005
$t_{1/2(el)}$	h	9.40 ± 0.36	9.10 ± 0.27
C_{max}	µg ml^{-1}	3.35 ± 0.16	3.28 ± 0.19
t_{max}	h	0.97 ± 0.03	0.99 ± 0.05
AUC	µg $ml^{-1}h^{-1}$	36.28 ± 1.34	34.25 ± 2.47
AUMC	µg $ml^{-1}h^{-2}$	440.87 ± 36.16	398.80 ±32.14
MRT	h	12.15 ± 0.27	11.64 ± 0.52

k_{ab}; K_{el} absorbtion and elimination rate constant after oral administration; $T_{1/2(ab)}$ absorbtion half life after oral administration; $T_{1/2(el)}$ elimination half life after oral administration; C_{max} maximum plasma concentration; T_{max} time to peak plasma concentration; AUC; area under serum concentration-time curve; AUMC area under moment curve; MRT mean residence time.

The disposition kinetics of doxycycline in Doxycycline Kela 75%® and Mebcodox 75%® following oral administration of 20 mg doxycycline base/kg.b.wt. revealed that the maximum blood concentration [C_{max}] were 3.35 and 3.28 µg/ml and attained at [T_{max}] of 0.97 and 0.99 hours, respectively. The mean ratio of C_{max} and AUC of the reference and tested formulations were within bioequivalence range and summarized in Table (3).

Table 3: Bioequivalence between Doxycycline Kela 75%® (Reference) and Mebcodox 75%® (Test) Formulations

Bioequivalence	C_{max}	AUC_{0-24}	$AUC_{0-\infty}$
Doxycycline Kela 75%® (Reference)	3.35±0.16	30.71±1.67	36.28±1.34
Mebcodox 75%® (Test)	3.28±0.19	29.39±1.28	34.25±2.47
Point estimate	0.97	0.95	0.94
Acceptable range	0.80-1.25	0.80-1.25	0.80-1.25
Conclusion	BE	BE	BE

BE-Bioequivalence

All the experimental chickens remained healthy during and after the study.

4. Discussion

The agar diffusion technique used here to determine serum and tissue concentrations of doxycycline is a dependable method, allowing pharmacokinetic data to be extrapolated to antibacterial activity with certainty (Santos et al., 1996; Vargas et al., 2008).

Antibiotics are widely used as veterinary drugs or as feed additives to promote growth (Yoshida et al., 1971; 1973; Yoshimura et al., 1991). The pharmacokinetics of doxycycline was reported in chickens following different routes of administrations (Anadon et al., 1994; Laczay et al., 2001; Ismail and El-Kattan 2004, Hantash et al., 2008 and El-Gendy et al., 2010). However, the current study was designed to investigate pharmacokinetics and bioequivalence of doxycycline of two oral formulations (Doxycycline Kela 75%® as a reference product and Mebcodox 75%® as a tested product) after oral administration in broiler chickens. Concentration of doxycycline in serum from 5 min up to 24 h exceeds the MIC against sensitive micro-organisms. The concentration was detected up to 24 hours in the serum of chickens (The MIC of doxycycline for M. gallisepticum strains S6 was 0.125 µg/ml; Zhang et al., 2016).

The elimination half lives of doxycycline following oral administration varies with age between 10 and 12 h (Pashov and Kamelov, 1994; Hantash et al., 2008). However, these values are notably different from the $T_{0.5(el)}$ values of 3.64 to 4.75 h reported by Anadon et al. (1994), Atef et al. (2002) and El-Gendy et al. (2010). In the present study a $T_{0.5(el)}$ of 9.40 and 9.10 h for Doxycycline Kela 75%® and Mebcodox 75%®, respectively; was determined and this may be in part explained by the use of an oral bolus dose.

It has been postulated that maximum efficacy in a clinical setting with doxycycline is achieved when serum concentrations of the drug are barely at or above the MIC level for the pathogen in question, for as long as possible within the dosing interval (Craig, 1998; Prescott et al., 2000). Values of MIC that can be adopted in this experiment can be categorised as susceptible for Mycoplasma gallisepticum (0.2 µg/ml: Takahashi and Yoshida, 1989; Stipkovits et al., 2004) and less susceptible for Escherichia coli (1-4 µg/ml: Cunha et al., 1982). Additionally, Notari (1987) suggested a MTC from 0.5 to 1µg/ml for this antibacterial drug and a similar value has been advanced for broilers (Hantash et al., 2008).

As pharmacokinetic-pharmacodynamic (PK/PD) surrogate indices (AUIC, AUC/MIC, and Cmax/MIC) for measuring the antibiotic efficacy are only well established for β-lactams, quinolones and aminoglycosides (Toutain et al., 2002), an additional work is needed for others antibiotics including doxycycline. In addition, owing to the high variations in MIC of sensitive veterinary pathogens, it is more important that doxycycline dosage regimens be calculated according to the sensitivity of the individual pathogen, site of infection and clinical response, than by following a percent dosage regimen.

Bioequivalence study is a test to assure the clinical efficacy of a generic versus brand drugs (Chen et al., 2001). Bioequivalence refers to a comparison between generic formulations of a drug, or a product in which a change has been made in one or more of the ingredients or in the manufacturing process, and a reference dosage form of the same drug. This study shows that the bioequivalence ratio for mean AUC_{0-24}, $AUC_{0-\infty}$ and C_{max} (T/R) of Mebcodox 75%® versus the reference products (Doxycycline Kela 75 %®) were 0.97, 0.95 and 0.94 respectively. These values were within the recommended range at the level of 90% confidence interval, 0.80 – 1.25 (Walker et al., 1992). The two formulations of doxycycline orally tested in this experiment could therefore be considered bioequivalent.

5. Conclusions

Based on the above pharmacokinetic and statistical results that calculated in the current study, we concluded that Mebcodox 75%® which manufactured by Boston Company, Mebco Vet Division, Egypt is bioequivalent to Doxycycline Kela 75%® which manufactured by Kela Veterinaria NV, Belgium and both products can be used as interchangeable drug in veterinary medicine practice especially in poultry.

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