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Original Article

PHARMACOKINETICS, BIO-EQUIVALENCE AND TISSUE RESIDUES OF TWO ORAL COLISTIN FORMULATIONS IN BROILER CHICKENS

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ABSTRACT

Objective: The present study was carried out to investigate and provide an overview of the pharmacokinetics, bio-equivalence and tissue residues of colistin in two oral tested products, BAC-Liquido® (Interchemi Co.) and Coline-L® (Medmac Co.) in healthy broiler chickens.

Methods: The comparative pharmacokinetics, bio-equivalence, blood and tissue residues of BAC-Liquido® and Coline-L® in broiler chickens was studied after oral administration of both products in a dose of 100.000 IU colistin base/kg. b. wt once daily for 5 consecutive days.

Results: Colistin in both products obeyed a two compartments open model following I. V administration. The disposition kinetics of BAC-Liquido® and Coline-L® following oral administration of 100.000 IU colistin base/kg. b. wt revealed that the maximum blood concentration [$C_{max.}$] were 5.10 and 4.95 µg/ml and attained at [$t_{max.}$] of 5.90 and 6.40 h, respectively. Colistin in BAC-Liquido® and Coline-L® was eliminated with half-lives [$t_{1/2\beta}$] equal to 3.15 and 2.89 h, respectively. The mean systemic bioavailability of colistin in BAC-Liquido® and Coline-L® following oral administration in healthy chickens was 3.75 and 4.05%, respectively. The blood (µg/ml) and tissue (µg/g) residues of Coline-L® and BAC-Liquido® following repeated oral administrations showed that liver, kidney; lung, breast, and thigh muscles contained the limited colistin residues. Colistin in both preparations was completely disappeared from all tissues at 24 h following the last oral dose (except liver 48 h).

Conclusion: It was concluded that Coline-L® is bioequivalent to BAC-Liquido® since $C_{max\ test}/C_{max\ reference}$ and $AUC_{test}/AUC_{reference}$ ratios were 0.97 and 1.06, respectively. Chickens should not be slaughtered for human consumption within treatment and could be consumed after the discontinuation of the treatment (except liver, withdrawal time 48 h) of either BAC-Liquido® or Coline-L®.

Keywords: Pharmacokinetics, Colistin, Broiler chickens, Bioavailability, Tissue residue.

INTRODUCTION

Antibiotics are normally administrated via feed or drinking water by veterinarians for therapy, prophylaxis and as a growth promoter in chickens. As a result, there is concern that residues of antibiotics may be retained in tissues from treated birds. Therefore, it is essential to obtain data for the target tissues for these drugs in chickens [1, 2].

Colistin, also known as polymyxin E, is a polypeptide antibiotic produced in culture broth of the aerobic spore-forming rod Bacillus Polymyxa Var. colistinus that can bind to membrane phospholipid of gram-negative bacteria to produce its strong effect against bacteria such as Escherichia coli, Pseudomonas aeruginosa, Bacillus, Salmonlla and Hemophilus [3]. In recent years, many bacterial strains developed strong resistance toward multiple drugs and antibiotics. Under such circumstances, colistin sulfate provides an effective treatment alternative to combat gram-negative bacteria that are sensitive to this antibiotic. Colistin sulfate binds to the bacterial lipopolysaccharide outer membrane and bacterial endotoxins leading to inhibition of the production and deactivation of bacterial endotoxins. Colistin sulfate is a chemical that is very effective in treating bacterial infections, especially those caused by multiple-drug-resistant gram-negative bacteria [4].

The ability of each antibiotic in this group to kill these bacteria varies [5]. Polymyxins B and E (colistin) are not absorbed from the intestine [6]. Previous studies showed no effect on reproductive ability, developmental toxicity or gene toxicity for colistin [7]. 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 [8].

The main purpose of this study is to investigate and provide an overview of the pharmacokinetics, bioequivalence and tissue residues of colistin in two oral tested products, BAC-Liquido® and Coline-L® in healthy broiler chickens.

MATERIALS AND METHODS

Drugs

BAC-Liquido®: is manufactured by Ascor Chimici, Italy. It is dispensed as an oral solution. Each one ml contains colistin sulphate 2,400,000 IU.

Coline-L®: is manufactured by Medmac, Amman, Jordan. It is dispensed as an oral solution. Each one ml contains colistin sulphate 2,400,000 IU.

Experimental design

Fifty clinically normal Hubbard chickens of 2 mo age, weighing 2000-2250 g were selected from Tanta Poultry Farm, Egypt. They were kept individually in cages, within a ventilated, heated room (20 °C) and 14 h of daylight. They received a standard commercial ration free from any antibiotics for 30 d before starting the experiments (to withdraw any antibiotic residues) and water *ad Libitum*. All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Cairo University.

Pharmacokinetics and Bioequivalence study

Twenty clinically normal Hubbard chickens were used to study the pharmacokinetics of BAC-Liquido® and Coline-L®after oral administration. Chickens were divided into two groups. The first group (10 chickens) was used to study the pharmacokinetics and bioequivalence of BAC-Liquido®. The 2nd group (10 chickens) was used to study the pharmacokinetics and bioequivalence of Coline-L®. Each chicken in both groups was injected intravenously with 25.000 IU colistin base/kg. b. wt (from colistin methanesulphonate, Sigma Aldrich®). Chickens were left for 15 d to ensure complete excretion of the antibiotic from their bodies. Then chickens from both groups

were administered orally (intra-crop) with BAC-Liquido® and Coline-L®in a dose of 100,000 IU colistin base/kg. b. wt, respectively.

Tissue residues study

Thirty clinically normal Hubbard chickens of 2-3 mo age, weighing about 2200-2450 g, were selected randomly from Tanta Poultry Farm, Egypt. Chicken was fed on a balanced ration free from antibiotic for 2 w (to withdraw any antibiotic residues) and water *ad Libitum*.

Tissue residue of colistin in BAC-Liquido® (3rd group = 15 chickens) and Coline-L® (4th group = 15 chickens) were determined following repeated oral administrations of 100.000 IU colistin base/kg. b. wt for BAC-Liquido® and Coline-L®, respectively once daily for five consecutive days. After the end of the fifth day of repeated oral administrations, three chickens were slaughtered at 24, 48, 72, 96 and 120 h for both groups, respectively.

Blood and tissue sample

One ml of blood was collected from wing vein after a single intravenous or oral administration of both drugs (groups 1 and 2) at intervals of 5, 15, 30 min, 1, 2, 4, 6, 8, 12 and 24 h. Blood samples were collected in dry centrifuge tubes. Serum was separated by centrifugation (2000 r. p. m./10 min) and stored at-20 °C until colistin assay.

After the end of the fifth day of repeated oral administrations of BAC-Liquido® and Coline-L®, three chickens were slaughtered at 24, 48, 72, 96 and 120 h, from each slaughtered chicken, blood, lung, liver, kidney, and muscles were taken for drug assay. Samples were frozen and stored at-20 °C until colistin assay.

Analytical procedure

Arret *et al.,* 1971 [9] described and modified by Tsai and Konda, 2001 [10]. a cylinder plate diffusion assay technique which used with a single layer of medium agar II (Difco). About 1 ml of the spore suspension of *Bordetella bronchiseptica* (ATCC 4617) obtained from the Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt. The organism was added to 100 ml agar II (at 55-60 °C). The mixture was shaken thoroughly till complete mixing of the test organism with the agar.

Petri dishes (120 X 20 mm) with the flat ad even bottoms were placed on a levels glass plate, and about 25 ml of inoculated medium was added to each dish by using a sterile cylinder (25 ml capacity) to form a thin layer of uniform thickness. After complete solidification, six wells were made on the surface of inoculated agar using stainless steel cylinder with sharp edges (10±0.1 mm length, 8±0.1 mm outside diameter, and 6±0.1 mm inside diameter) careful vertical punching creates wells that were clean and symmetric. A paper grid system under the plate facilitates the even spacing of the wells that allows for the triplicate determination of standards and samples.

Three plates were used for each sample; three wells on each plate were filled with the reference concentration (5 µg of colistin/ml free serum or phosphate buffer). The other three wells were filled with the sample (serum or organ homogenate). The plates were incubated at 37 °C for 16–18 h. The diameter of each inhibition zone was measured (mm) and plotted in semilogarithmic paper using concentration in (µg/ml) and the diameter of the zone of inhibition (mm). The standard curves were drawn through these points. The serum and tissue residues concentrations were obtained.

Pharmacokinetic and statistical analysis

The pharmacokinetic parameters of colistin were calculated by using a non-compartmental software program (WinNonlin® software, version 5.2, Phar sight Corporation, NC, USA). The area under the serum concentration–time curve (AUC) was calculated using the trapezoidal rule with extrapolation to infinity. The maximum concentration (C_{max}) and the corresponding peak time (t_{max}) were determined by the inspection of the individual drug serum concentration–time profiles. The slope of the terminal phase of the time–concentration curve was determined by linear regression and converted to an elimination half-life ($t_{1/2\beta}$) by multiplying the reciprocal by 0.693.

Bioavailability

The rate of absorption after oral administration was determined by comparing the area under the serum concentration-time curve (AUC) oral with that obtained following intravenous injection (AUC) i. v. in the same chicken.

$$\textbf{Bioavailability} = \frac{(AUC)_{oral} \times D_{i.v}}{(AUC)_{i.v} \times D_{oral}} \times 100$$

Where: D_{iv} = Dose of intravenous injection.

D_{oral} = Dose of oral administration.

Bioequivalence

The following equation according to FDA regulation [8] was performed to prove that the tested product is bioequivalent to the reference product in the study:

$$\frac{AUC_{(test)}}{AUC_{(reference)}} \text{ or } \frac{C_{max\,(test)}}{C_{max\,(reference)}}$$

Where: this ratio ranges from 0.85 to 1.25, this indicates that the test product Coline-L® is bioequivalent to the reference product BAC-Liquido®.

Data were expressed as mean±SE and were statistically analyzed using analysis of variance. Mean comparisons were performed using Tukey's test. The differences were considered significant when $p<0.05$. These calculations were performed using Prism 5.0 (GraphPad).

Table 1: Pharmacokinetic parameters of colistin in BAC-Liquido® and Coline-L® following IV administration of 25.000 IU colistin base/kg. b.wt in broiler chickens (n = 10), mean±SE

Parameter	Unit	G1 BAC-Liquido®	G2 Coline-L®
C °	µg/ml	230.00±4.50	225.00±6.60
$t_{1/2\alpha}$	h	0.16±0.08	0.11±0.06
V_c	L/kg	1.50±0.18	1.70±0.25
$V_{d(area)}$	L/kg	2.40±0.60	2.70±0.75
V_{dss}	L/kg	2.75±0.45	2.45±0.60
K_{12}	h^{-1}	0.75±0.06	0.95±0.08
K_{21}	h^{-1}	0.67±0.08	0.71±0.07
$t_{1/2\beta}$	h	0.50±0.08	0.45±0.06
Cl_B	L/kg/h	83.37±4.45	87.77±6.40
$AUC_{0-\infty}$	µg. h/ml	436.30±12.20	430.90±7.00

C ° = Drug concentration in serum at zero time immediately after a single intravenous injection; $AUC_{0-\infty}$ = area under the concentration-time curve from zero up to∞ with extra polation of the terminal phase; $t_{½\beta}$ = half-life of the elimination; V_c= volume of the central compartment; Vd_{area}= Volume calculated by the area method; V_{dss} = apparent volume of distribution at steady-state; Cl_B = clearance from the body. K_{12} = First order transfer rate constant for drug distribution from central to the peripheral compartment; K_{21} =First order transfer rate constant for drug distribution from peripheral to central compartment.

RESULTS

The mean serum concentration-time curve of colistin in BAC-Liquido® and Coline-L® following I. V and oral administration is plotted and presented graphically in fig. (1, 2). The pharmacokinetic parameters of colistin in BAC-Liquido® and Coline-L® following I. V. administration of 25.000 IU colistin base/kg. b. wt in broiler chickens were calculated and showed in the table (1). The pharmacokinetic parameters of colistin in BAC-Liquido® and Coline-L® after oral administration of 100.000 IU colistin base/kg. b. wt in broiler chickens were calculated and showed in the table (2).

The disposition kinetics of colistin in BAC-Liquido® and Coline-L® following oral administration of 100.000 IU colistin base/kg. b. wt revealed that the maximum blood concentration [$C_{max.}$] were 5.10 and 4.95 µg/ml and attained at [$t_{max.}$] of 5.90 and 6.40 h, respectively. Colistin in BAC-Liquido® and Coline-L® was eliminated with half-lives [$t_{1/2\beta}$] equal to 3.15 and 2.89 h, respectively. The mean systemic bioavailability of colistin in BAC-Liquido® and Coline-L® following oral administration in broiler chickens was 3.75 and 4.05%, respectively. The oral bioavailability of BAC-Liquido® and Coline-L® indicated a poor absorption from GIT which indicated that both formulations are advised to be given orally in the case of acute bacterial infections in GIT.

Blood and tissue residues of colistin in BAC-Liquido® and Coline-L® in slaughtered chickens following repeated oral administrations of 100.000 IU colistin base/kg. b. wt once daily for 5 consecutive days are recorded in the table (3). The represented data revealed a poor spread distribution of colistin in both BAC-Liquido® and Coline-L® in lung, liver, kidney, and muscles (Breast and Thighs).

Table 2: Pharmacokinetic parameters of colistin in BAC-Liquido® and Coline-L® following oral administration of 100.000 IU colistin base/kg. b. wt in broiler chickens (n = 10), mean±SE

Parameter	Unit	G1 BAC-Liquido®	G2 Coline-L®
k_{ab}	h^{-1}	2.86±0.80	3.44±0.50
$t_{1/2\ ab}$	h	0.24±0.09	0.20±0.06
$t_{1/2\beta}$	h	3.15±0.35	2.89±0.40
$t_{max.}$	h	5.90±0.80	6.40±0.95
$C_{max.}$	µg/ml	5.10±0.35	4.95±0.80
AUC_{0-t}	µg. h/ml	16.40±1.40	17.45±2.00
Bioavailability	%	3.75±0.75	4.05±0.60
Bio-Equivalent			
AUC	Ratio		1.06
$C_{max.}$	Ratio		0.97

C_{max} = maximal concentration; t_{max} = when the maximal serum concentration is reached; AUC_{0-t} = area under serum concentration time curve; $t_{1/2\beta}$ = Elimination half-life; K_{ab} = first-order absorption rate constant; $t_{1/2\ ab}$ = The absorption half-life (h).

Table 3: Blood levels (µg/ml) and tissue concentrations (µg/g) of colistin in BAC-Liquido® and Coline-L® following repeated oral administrations of 100.000 IU colistin base/kg. b. wt once daily for five consecutive days in broiler chickens (n=3), mean±SE

Blood and Tissues	Time after the last dose (h)									
	24		48		72		96		120	
	BAC-Liquido®	Coline-L®	BAC-Liquido®	Coline-L®	BAC-Liquido®	Coline-L®	BAC-Liquido®	Coline-L®	BAC-Liquido®	Coline-L®
Blood	N. D	N. D	N. D	N. D	N. D	N. D	N. D	N. D	N. D	N. D
Lung	N. D	N. D	N. D	N. D	N. D	N. D	N. D	N. D	N. D	N. D
Liver	3.60	3.30	0.30	0.15	N. D	N. D	N. D	N. D	N. D	N. D
Kidney	N. D	N. D	N. D	N. D	N. D	N. D	N. D	N. D	N. D	N. D
Breast and Thigh Muscles	N. D	N. D	N. D	N. D	N. D	N. D	N. D	N. D	N. D	N. D

N. D = Not detected, After the end of the fifth day of repeated oral administrations, three chickens were slaughtered at 24, 48, 72, 96 and 120 h.

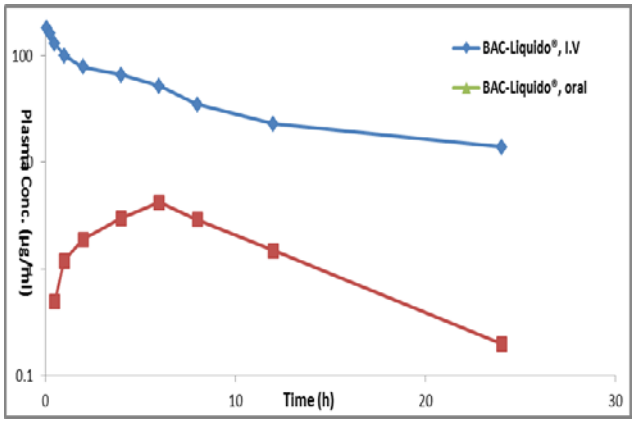

Fig. 1: Semilogarithmic plot showing the serum concentrations-time profile of colistin in BAC-Liquido® following intravenous and oral administration in broiler chickens (n=10)

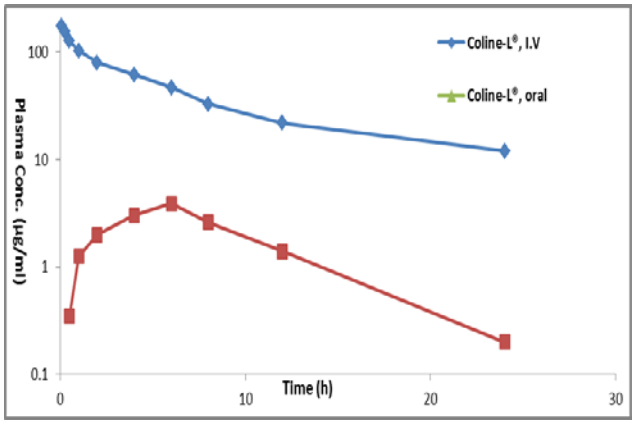

Fig. 2: Semilogarithmic plot showing the serum concentrations-time profile of colistin in Coline-L® following intravenous and oral administration in broiler chickens (n=10)

DISCUSSION

Antibiotics are widely used as veterinary drugs or as feed additives to promote growth. Some studies had induced pharmacokinetic data in poultry [11-15].

Colistin in both formulations following I. V. administration obeyed a two compartments open model, this indicated that colistin distributed in the chickens in two compartments; a central one which represents blood and highly perfused organs (kidney–liver–spleen–heart) and a 2nd peripheral compartment which represented by skin and connective tissues. Following I. V. administration in a dose of 25.000 IU colistin base/kg. b. wt, colistin is obeyed a two-compartment open model with high volume of distribution (exceeded than one L/kg) calculated by extrapolation [V_{dB}] and steady state [V_{dss}] method are factors made colistin is highly distributed in all body tissues; a factor revealed that colistin, when given by IV injection, is the drug of choice for attacking the systemic infections caused by sensitive organisms. [16] reported that I. V. administration of 5 mg colistin/kg. b. wt in ewes resulted in a serum half-life of 2.7–4.3 h and a V_d of 1.29 L/kg. There is limited information on the mechanism by which colistin is formed from colistin methanesulphonate *in vitro* and *in vivo* [17, 18]. A statistically significant difference was not found between the calculated pharmacokinetic parameters in the investigated groups, these results were showing the bioequivalence of the two formulations according to the criteria established by FDA [8]. 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 [19].

In man, absorption of polymyxins from the gastrointestinal tract is slow and limited so that ordinary oral doses do not produce detectable plasma concentrations. The same may be true for other monogastric animals. Under normal conditions, less than 0.5% of the oral administered dose is absorbed from the gastrointestinal tract [20].

EMEA 2002 [21] investigated that hens were orally given colistin sulphate at dosages of 25 mg and 50 mg/kg body weight, the serum level in the 25 mg group was highest after 1 hour (1.5 mcg/ml); thereafter, it decreased by time and was no longer detectable 6 h post administration. In the bile, the highest colistin concentration was measured at the first hour (2.5 mcg/g), however, after 6 h no concentrations were detectable. The serum level in the 50 mg group was highest after 2 h (10.2 mcg/ml), decreasing gradually thereafter and 8 h post administration, no concentrations were detectable. In the bile, colistin showed the highest concentrations 2 h post administration, however, 8 h post administration, no concentrations were detectable. Also, EMEA 2002 [21] investigated that the pharmacokinetic data in the target species confirmed that colistin sulphate was poorly absorbed after oral administration to calves, pigs and rabbits and serum concentrations were generally undetectable in the species. In chickens, residues in serum were detectable for up to 6 h after administration in the drinking water.

Colistin could not be determined in all tissues tested in all time intervals after the last dose. This indicated that colistin is not absorbed after oral route in concentration could be detected [22]. In the same direction, [6] investigated that colistin residues were not detected after the drug administration by the oral route but could be detected in the yolk until 8 d after intramuscular injection.

EMEA 2002 [21] and FAO/WHO 2006 [2] recorded that residues of colistin could be detectable in serum for up to 24 h after intramuscular or intravenous administration to calves and dairy cows. In calves, bioavailability approached 100% after intramuscular administration. In ewes, peak serum concentrations of 8-20 µg/ml were achieved 2 h after intramuscular injection. Residues in eggs from hens given colistin sulphate in the drinking water were below the limit of detection of the analytical method. Significant residues were found up to 8 d in eggs following intramuscular injection to hens.

In the present work, the tissue residues of colistin in Colin-L® and BAC-Liquid® at all times were below the MRLs approved by [2].

CONCLUSION

Based on the above pharmacokinetic and statistical results that calculated in the current study, we concluded that Coline-L®is bioequivalent to BAC-Liquido® since $C_{max\ test}/C_{max.\ Reference}$ and $AUC_{test}/AUC_{reference}$ ratios were 0.97 and 1.06, respectively. In addition, chickens should not be slaughtered for human consumption within treatment and could be consumed after the discontinuation of the treatment (except liver, withdrawal time 48 h).

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CONFLICT OF INTERESTS

Declare none

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