e ISSN 2277 - 3290
Print ISSN 2277 - 3282

Journal of Science

Veterinary

www.journalofscience.net

PHARMACOKINETICAL INTERACTIONS OF LINCOMYCIN AND AMPROLIUM IN BROILER CHICKENS

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ABSTRACT

Pharmacokinetic parameters and bioavailability of lincomycin following a single intravenous and oral administrations were determined in broiler chickens. Effect of amprolium on the disposition kinetics and tissue residues of lincomycin following repeated oral administrations was also investigated. Following a single intravenous injection of lincomycin, 20 mg/kg.b.wt., in normal broiler chickens, plasma concentration-time curve was best described by a two-compartments open model with elimination half-life ($t_{0.5\beta}$ = 2.93±0.014 h), volume of distribution (V_{dss} = 1.76±0.014 L/kg) and total clearance of the drug (CL_{tot} = 0.457±0.004 L/kg/h). Following a single oral administration, the maximum plasma concentration was 7.08±0.16 µg/ml, reached at maximum time of 1.16±0.02 h. The mean systemic bioavailability following oral administration was 71.32± 2.62%. Following repeated oral administration in normal chickens, highest plasma concentration peaked after one hour of each oral dose. Amprolium resulted in a significant decrease in maximum plasma concentration (C_{max} = 7.29 ± 0.204 µg/ml) compared with lincomycin alone (C_{max} = 8.13 ± 0.244 µg/ml). Amprolium resulted in a significant decrease in tissue residues of lincomycin. It is concluded that the administration of amprolium before lincomycin in broiler chickens would altered the pharmacokinetic profile of lincomycin.

Keywords: Pharmacokinetics, Interaction, Lincomycin, Amprolium, Chickens.

INTRODUCTION

Pharmacokinetic drug interactions are of great clinical importance in veterinary practice. Drugs have the same metabolic pathway usually show drug interactions in its concomitant administration [1].

Lincosamides is a group of monoglycoside antibiotics containing amino-acid like side chain. It is a miscellaneous group of protein inhibiting antimicrobials with activities similar to members of the macrolide group of antibiotics. Lincomycin is a member of the lincosamide antibiotics, mainly active against *Staphylococci*, *Streptococci* and anaerobic bacteria including *Bacteroides fragilis* [2]. It is used alone or in combination with other drugs in poultry for oral treatment of bacterial enteric infections, control of respiratory infections and growth enhancement.

The pharmacokinetics of lincomycin have been determined for a variety of animals including sheep [3], dogs [4], calves [5, 6], pigs [7], chickens [8, 9], goats [10], and cats [11]. Amprolium is a thiamine analogue used in treatment and prevention of coccidiosis in poultry and rabbits [12]. The aim of this study is to investigate the effect of amprolium pretreatment on the pharmacokinetics and tissue residues of lincomycin in broiler chickens. The pharmacokinetic profiles of the lincomycin, following a single intravenous and oral administrations in normal chickens were determined. Bioavailability of lincomycin in normal chickens was calculated. The pharmacokinetic profiles as well as tissue residues of lincomycin following repeated oral administrations in normal chickens and in chickens previously given amprolium were achieved.

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MATERIAL AND METHODS

Drugs:

Lincomycin: (LINCOPHARM) ®

It was obtained as a powder from Dopharma B.V., Netherlands, under trade name of LINCOPHARM ®. The vial contains 150 g each one gram contains lincomycin hydrochloride 200 mg of the active lincomycin.

Amprolium: (Amprolium 20%) ®

Amprolium was obtained as a water-soluble powder 20% under trade name of Amprolium 20% ® from Adwia company, Cairo, Egypt.

Experimental design: Thirty-seven clinically normal Hubbard chickens of 2 months age weighing about 1600-2000 grams were used in this investigation. Chickens were fed on balanced ration free from antibiotics for two weeks to ensure complete excretion of any drugs from their bodies. Water free from antibacterial additives was *ad-libitum*.

Chickens were divided into three groups:

Group (1): This group included seven normal chickens. Each bird was injected with lincomycin 20 mg/b.wt. intravenously into the left wing vein. These chickens were left for fifteen days to ensure complete excretion of the tested drug from their bodies. Then the tested chickens were administered the same dose orally to calculate the systemic oral bioavailability.

Group (2): This group included 15 normal chickens for assay of tissue residues and kinetic parameters of lincomycin in normal chickens. Chickens were administered lincomycin (20 mg/b.wt.) orally, twice daily for five consecutive days. Twenty-four hours after the last dose of administration, three chickens were slaughtered and then after 48, 72, 96 and 120 hours.

Group (3): This group included 15 normal chickens for assay of tissue residues and kinetic parameters of lincomycin in chickens pre-treated with amprolium. Each bird was administered amprolium (30 mg/b.wt.) orally once daily for five consecutive days, 2 hours after the last dose, to ensure that amprolium reaches its maximum concentration, the chickens were administered lincomycin (20 mg/b.wt.) orally, twice daily for five consecutive days. Twenty-four hours after the last dose of administration, three chickens were slaughtered and then after 48, 72, 96 and 120 hours.

Collection of samples

Blood samples: Blood samples were collected from all groups of chickens in heparinized test tubes, and centrifuged at 2500 rpm for 30 minutes to separate the plasma for the estimation of the drug. Blood samples were taken from either right or left wing vein of chickens were collected after 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after a single intravenous and oral (single & repeated) administrations in all groups. All plasma samples were frozen at –20°C until assay.

Tissue samples

After the end of the repeated oral administration of lincomycin in normal chickens (group 2), and in chickens pre-treated with amprolium (group 3), three chickens were slaughtered after 24, 48, 72, 96 and 120 hours. Tissue samples from brain, heart, gizzard, liver, spleen, kidney, breast muscles, thigh muscles, fat and skin were taken from each slaughtered bird for lincomycin assay. Samples were frozen and stored at –20°C until assayed.

Analytical procedures

Lincomycin was assayed in plasma and tissues of chickens by modified spectrophotometric method of [13] by using a double beam UV- visible spectrophotometer (T60U, United Kingdom) in the central lab., that follow Center of Excellence for Scientific Research (CESR) - Faculty of Veterinary Medicine- Benha University. A stock solution (100 µg / ml) of lincomycin in distilled water or plasma (antibiotic free) of normal chickens were prepared. Standard concentrations were obtained by further dilution to obtain concentrations varying from 1.25, 2.5, 5, 10, 25, 50 to 100 µg lincomycin per milliliter-distilled water or plasma. Optical densities of the drug molecule of different concentrations were read at 196 nm, using a quartz cuvette by a double beam UV-visible spectrophotometer. Concentrations of the drug at different time intervals were obtained and then plotted against optical densities on a graph paper to obtain standard curves.

The pharmacokinetic parameters were calculated by WinNonlin, version (2.1) and other parameters according to [14]. All statistical analysis was carried out according to [15].

Assay of tissue samples

Three milliliters of distilled water were added to one gram of the obtained tissue sample and homogenized in a porcelain mortar by the aid of sterile sand. The homogenate was left in the refrigerator overnight then centrifuged. The supernatant was taken and subjected to the same procedures for assay in plasma samples. Concentrations of the drug at different time intervals were obtained from the standard curve of lincomycin in distillated water prepared previously and expressed as µg / gm of the tissue.

RESULTS

The semi logarithmic plasma concentration-time curve of lincomycin in normal broilers, after a single intravenous injection of 20 mg/kg b.wt. showed that the drug obeyed a two- compartments open model. Lincomycin could be detected therapeutically for 8 hours post intravenous injection (Fig.1).

The semi logarithmic plasma concentration-time curve of lincomycin in normal broilers, after a single oral administration of 20 mg/kg.b.wt. is depicted in fig. (2).

The disposition kinetics of lincomycin following a single intravenous and oral administration were recorded in table (1).

Repeated oral administration of 20 mg/kg b.wt twice daily for five consecutive days in normal chickens and in that previously given amprolium 30 mg/kg.b.wt. once daily for five consecutive days revealed a lower significant plasma concentration of lincomycin in chickens previously given amprolium when compared with normal chickens at most times of sampling (Figures 3&4).

The pharmacokinetic parameters of lincomycin as well as tissue residues after repeated oral administration in normal chickens were compared to that previously given amprolium (Tables 2&3).

[1]A & B, Zero time serum drug concentration intercepts of biphasic intravenous disposition curve. The coefficient B is based on the terminal exponential phase (µg/ml); α & β, Hybrid rate constant of biphasic intravenous disposition curve values of α and β are related to the slopes of distribution and elimination phase respectively, of biexponential drug disposition curve (h-1); AUC, Total area under the serum drug concentration versus time curve from t = 0 to t = α after administration of a single dose; C°, Drug concentration in the serum at zero time immediately after a single intravenous injection (µg/ml); C max, Maximum serum concentration of drug in blood after extra vascular administration (µg/ml); Cl tot, The total clearance of a drug, which represents the sum of all clearance processes in the body (ml/kg /min); K12 , First - order transfer rate constant for drug distribution from central to peripheral compartment (h^{-1}); K21 , First order transfer rate constant for drug distribution from peripheral to central compartment (h^{-1}); t 0.5(α), Distribution half - life (h); t 0.5(β), Elimination half - life (h); t max , The time at which the maximum concentration of drug was reached after extravascular administration (h); Vdss, The apparent volume of distribution which was calculated by Steady - state method (ml/kg).

Table 1. Pharmacokinetic parameters of lincomycin following a single intravenous and oral administration of 20 mg/kg b.wt. in normal chickens (n=7).

Parameter [1]	Unit	Intravenous $\overline{X}$ ± S.E.	Oral $\overline{X}$ ± S.E.
B.wt	Kg	1.870±0.053	2.093±0.049
C^{o}	µg/ml	28.66±0.219	12.33 ± 0.40
A	µg/ml	19.13±0.191	3.20 ± 0.01
α	h^{-1}	5.087±0.085	2.24 ± 0.01
$t_{0.5(\alpha)}$	h	0.137±0.002	0.310 ± 0.01
K_{12}	h^{-1}	2.82±0.054	-
K_{21}	h^{-1}	1.85±0.035	-
V_{dss}	L/kg	1.76±0.014	-
B	µg/ml	9.53±0.108	9.13 ± 0.043
β	h^{-1}	0.236±0.001	0.206 ± 0.01
$t_{0.5(\beta)}$	h	2.93±0.014	3.35 ± 0.011
K_{10}	h^{-1}	0.651±0.007	-
$t_{0.5(K_{10})}$	h	1.066±0.011	-
C_{max}	µg/ml	-	7.08 ± 0.16
T_{max}	h	-	1.16 ± 0.02
Cl_{tot}	L/kg/h	0.457±0.004	0.007 ± 0.003
MRT	h	3.87±0.020	-
AUMC	µg/ml/h	170.42±2.236	-
$AUC_{(oral)}$	µg/ml/h	-	31.49 ± 1.103
$AUC_{(i.v.)}$	µg/ml/h	44.18 ± 0.422	-
F	%	-	71.32 ± 2.62

Table 2. Pharmacokinetic parameters of lincomycin (μg/ml) following repeated oral administration of 20 mg/kg b. wt. twice daily for consecutive 5 days in normal chickens and in chickens previously given amprolium 30 mg/kg b.wt. once daily for 5 consecutive days (n=7).

`Parameter[1]	Unit	Dose First		Third		Fifth		Seventh		Ninth	
		linco ($\bar{X}$±SE)	linco +amp. ($\bar{X}$±SE)	linco. ($\bar{X}$±SE)	linco.+amp. ($\bar{X}$±SE)	linco. ($\bar{X}$±SE)	linco.+amp. ($\bar{X}$±SE)	linco. ($\bar{X}$±SE)	linco.+amp. ($\bar{X}$±SE)	linco. ($\bar{X}$±SE)	linco.+amp. ($\bar{X}$±SE)
C^o	μg/ml	10.03± 0.279	12.43± 0.361***	10.50± 0.294	12.10± 0.411***	10.77±0. 302	14.89± 0.521***	11.04±0. 320	13.59± 0.516***	11.13± 0.312	12.63± 0.467***
A	μg/ml	3.26± 0.098	3.25± 0.078	3.16± 0.095	4.93± 0.143***	3.16± 0.095	6.93± 0.208***	3.13± 0.094	5.64± 0.197***	3.11± 0.093	4.67± 0.182***
α	h^{-1}	2.14± 0.010	2.29± 0.060	1.53± 0.044	2.80± 0.081***	1.53± 0.044	2.87± 0.861*	1.51± 0.044	2.82± 0.102***	1.45± 0.042	3.92± 0.145***
$t_{0.5(\alpha)}$	h	0.324± 0.010	0.303± 0.009*	0.454± 0.014	0.248± 0.007***	0.454± 0.014	0.241± 0.009***	0.460± 0.014	0.246± 0.009***	0.475± 0.014	0.177± 0.006***
K_{01}	h^{-1}	1.51± 0.042	1.31± 0.046***	1.59± 0.045	1.34± 0.038***	1.55± 0.045	1.40± 0.039**	1.51± 0.045	1.36± 0.037**	1.40± 0.041	1.34± 0.040
$t_{0.5(01)}$	h	0.46± 0.013	0.528± 0.019**	0.436± 0.013	0.519± 0.015***	0.448± 0.013	0.495± 0.014**	0.445± 0.001	0.507± 0.014***	0.494± 0.0145	0.517± 0.018
K_{12}	h^{-1}	0.534± 0.016	0.480± 0.012**	0.515± 0.015	0.529± 0.014	0.539± 0.016	0.529± 0.015	0.535± 0.016	0.534± 0.015	0.545± 0.016	0.520± 0.016
K_{21}	h^{-1}	0.773± 0.022	0.605± 0.016***	0.842± 0.025	0.634± 0.017***	0.811± 0.023	0.696± 0.020***	0.787± 0.024	0.604± 0.017***	0.697± 0.021	0.598± 0.021***
T_{max} (calc.)	h	0.960± 0.027	1.03± 0.027*	0.970± 0.029	1.01± 0.028	0.970± 0.029	1.01± 0.025	0.981± 0.029	1.00± 0.028	0.992± 0.030	1.01± 0.028
C_{max} (calc.)	μg/ml	6.53± 0.19	5.60± 0.14***	6.76± 0.203	5.99± 0.168***	7.19± 0.216	6.33± 0.158***	7.55± 0.227	6.66± 0.200***	8.13± 0.244	7.29± 0.204**
B	μg/ml	6.77± 0.190	9.18± 0.275***	7.34± 0.206	7.17± 0.194	7.61± 0.221	7.96± 0.287	7.91± 0.237	7.95± 0.215	8.02± 0.241	7.96± 0.295
β	h-1	0.213± 0.006	0.208± 0.006	0.210± 0.006	0.220± 0.006	0.205± 0.006	0.210± 0.007	0.201± 0.006	0.213± 0.006	0.198± 0.006	0.212± 0.008
$t_{0.5\beta}$	h	3.25± 0.091	3.33± 0.100	3.30± 0.096	3.16± 0.088	3.38± 0.101	3.31± 0.113	3.44± 0.100	3.25± 0.124	3.51± 0.098	3.27± 0.118*
K_{10}	h-1	0.417± 0.012	0.420± 0.012	0.382± 0.012	0.420± 0.012**	0.387± 0.012	0.402± 0.011	0.386± 0.012	0.397± 0.012	0.413± 0.012	0.410± 0.012
$t_{0.5\ (K10)}$	h	1.66± 0.047	1.65± 0.048	1.81± 0.053	1.65± 0.132	1.79± 0.052	1.73± 0.047	1.79± 0.052	1.75± 0.051	1.67± 0.048	1.69± 0.047
CL_{tot}	L/kg/h	0.007± 0.0002	0.006± 0.0002	0.007± 0.0002	0.006± 0.0002	0.006±0. 0002	0.005± 0.0002	0.006± 0.0002	0.005± 0.0002	0.007± 0.0002	0.006± 0.0002
AUC	μg/ml/h	32.31± 0.910	28.75± 0.863**	34.81± 1.040	31.11± 0.871**	37.33± 1.120	33.30± 0.932**	39.60± 1.190	36.18± 1.090*	42.02± 1.260	38.55± 1.080*

*P<0.05 ** P<0.01 *** P<0.001

linco. = lincomycin amp. = amprolium

Table 3. Plasma (μg/ml) and tissue concentrations (μg/g) of lincomycin following repeated oral administration of 20 mg/kg b. wt. twice daily for 5 consecutive days in normal chickens and in chickens previously given amprolium 30 mg/kg b. wt. once daily for 5 consecutive days (n=3).

Tissue	Days							
	First		Second		Third		Fourth	
	Linco. ($\bar{X}$ ±S.E.)	Linco. + amp. ($\bar{X}$ ±S.E.)	Linco. ($\bar{X}$ ±S.E.)	Linco. + amp. ($\bar{X}$ ±S.E.)	Linco. ($\bar{X}$ ±S.E.)	Linco. + amp. ($\bar{X}$ ±S.E.)	Linco. ($\bar{X}$ ±S.E.)	Linco. + amp. ($\bar{X}$ ±S.E.)
Plasma	1.21± 0.09	1.09± 0.07	0.80± 0.07	0.63± 0.01**	0.46± 0.03	0.31± 0.02***	–	–
Brain	0.137± 0.01	0.64± 0.04***	1.01± 0.03	0.34± 0.06***	–	–	–	–
Heart	1.43± 0.06	0.88± 0.03***	1.04± 0.05	0.55± 0.03***	0.72± 0.03	0.22± 0.02***	0.44± 0.03	

Liver	2.13± 0.09	1.77± 0.04***	1.74± 0.05	1.13± 0.07***	1.26± 0.05	0.62± 0.02***	0.73± 0.04	–
Kidney	1.90± 0.08	1.55± 0.06***	1.52± 0.05	0.84± 0.04***	0.95± 0.04	0.55± 0.03***	0.55± 0.02	–
Spleen	1.75± 0.06	1.33± 0.06***	1.45± 0.05	0.82± 0.07***	0.88± 0.06	0.38± 0.04***	0.47± 0.02	–
Gizzard	1.59± 0.03	1.07± 0.06***	0.45± 0.02	0.35± 0.02***	0.33± 0.01	0.23± 0.03***	–	–
Breast muscle	0.68± 0.05	0.59± 0.02*	0.46± 0.02	0.37± 0.02***	0.27± 0.01	0.20± 0.01***	–	–
Thigh muscle	0.54± 0.05	0.44± 0.04*	0.39± 0.02	0.28± 0.02***	0.23± 0.01	0.19± 0.01**	–	–
Fat	1.19± 0.03	1.03± 0.04***	1.00± 0.03	0.65± 0.04***	0.61± 0.07	0.53± 0.03	0.35± 0.02	–
Skin	0.97± 0.05	0.73± 0.04***	0.73± 0.02	0.46± 0.02***	0.44± 0.01	0.26± 0.03***	0.28± 0.02	–

*P<0.05 ** P<0.01 *** P<0.001

Linco. = lincomycin amp. = amprolium

Figure 1. Semi-logarithmic graph depicting the time course of lincomycin (μg /ml) in plasma of normal broiler chickens following a single intravenous injection of 20 mg/kg b.wt. (n=7).

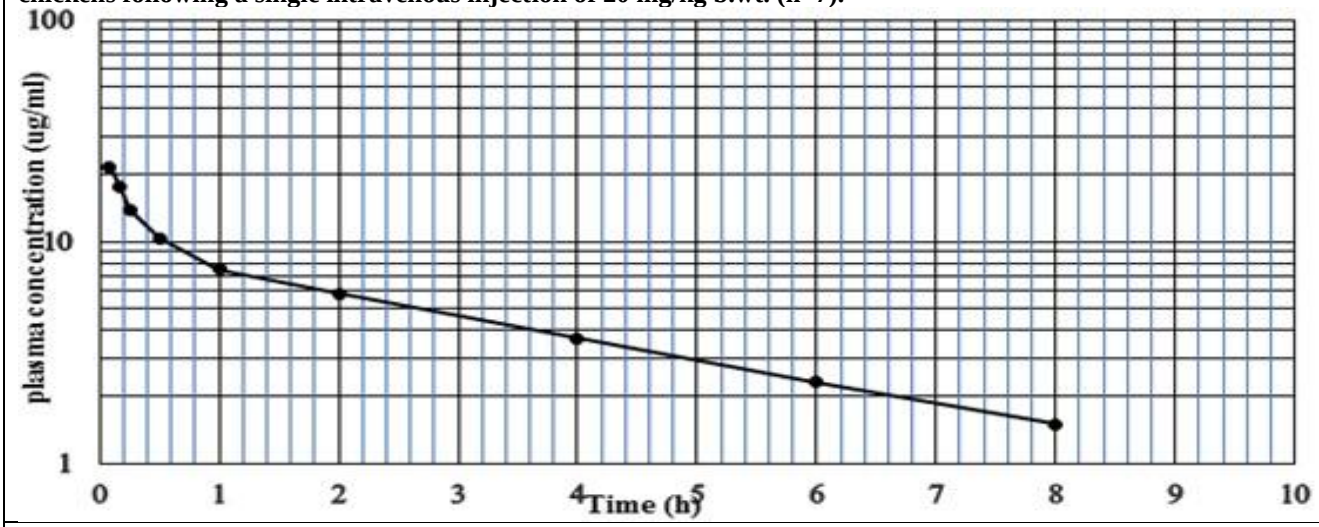


Figure 2. Semi-logarithmic graph depicting the time course of lincomycin (μg/ml) in plasma of normal broiler chickens following a single oral administration of 20 mg/kg b. wt. (n=7).

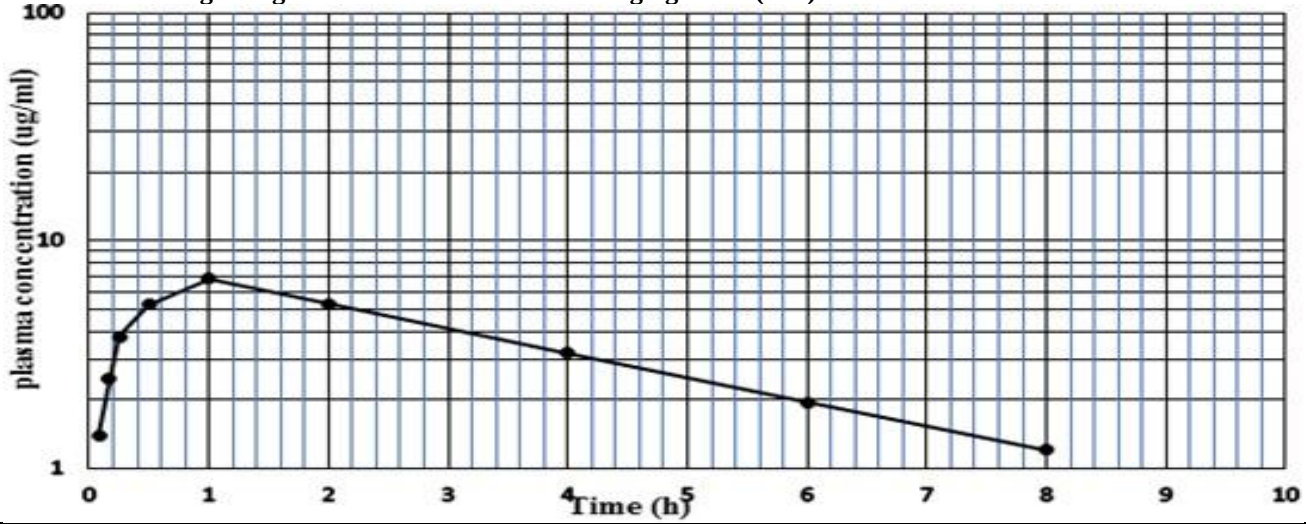


Figure 3. Semi-logarithmic graph depicting the time course of lincomycin (µg/ml) in plasma of normal chickens following repeated oral administration of 20 mg/kg b. wt. twice daily for 5 consecutive days (n=7).

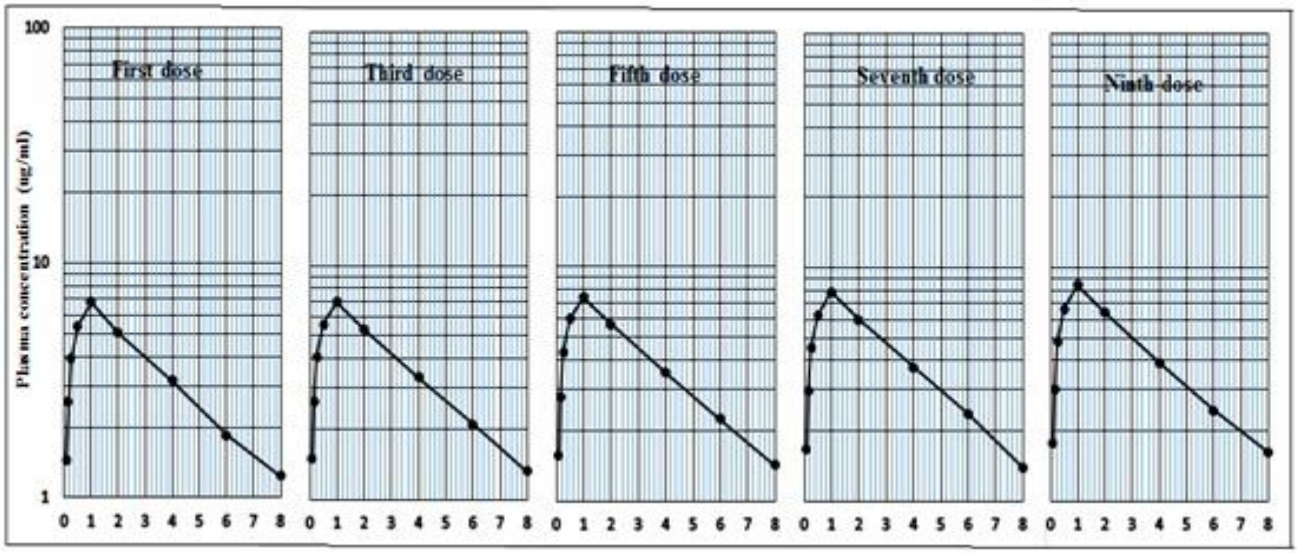
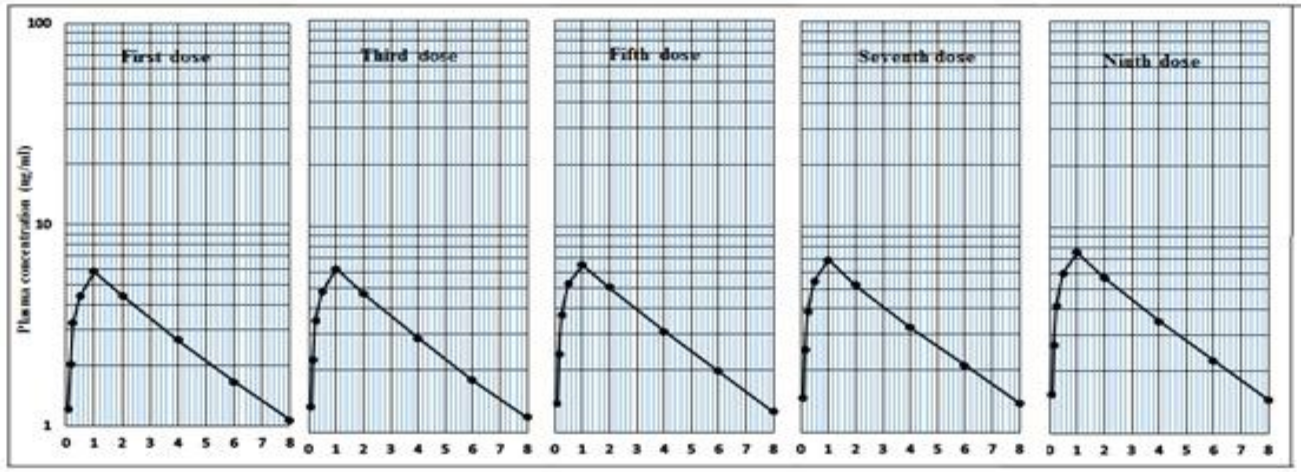


Figure 4. Semi-logarithmic graph depicting the time course of lincomycin (µg/ml) following repeated oral administration of 20 mg/kg b. wt. twice daily for 5 consecutive days in chickens previously given amprolium 30 mg/kg b. wt. once daily for 5 consecutive days (n=7).



DISCUSSION

The present investigation revealed that the plasma concentration–time curve of lincomycin decreased in a bi-exponential manner, indicating the presence of a distribution and an elimination phases and justifying the use of a two-compartment kinetic model for analyzing the data following a single intravenous injection of 20 mg/kg body weight in normal chickens. The plasma concentration time curve of lincomycin obeyed a two-compartments open model, a central compartment which represented by the blood and rapid equilibrating tissues (lung, liver, kidneys and spleen) and a peripheral compartment represented by slower equilibrating tissues. This result was agreed with that recorded in broiler chickens. [8, 9, 16, 17] This finding was also observed in goats [10], cats [11], and calves. [5, 6]

The plasma concentration of lincomycin following a single intravenous injection of 20 mg/ kg body weight in normal chickens decreased from 21.88 ± 0.099 µg /ml at 5 minutes to 1.51 ± 0.028 µg /ml at 8 hours post injection. This concentration was higher than the minimum inhibitory concentration value of lincomycin for Staphylococcus spp. (1.0 µg/mL) and for Streptococcus spp (0.4 µg/mL) [17]. The disposition kinetics of lincomycin following a single intravenous

injection revealed a distribution phase (a) equal 5.087±0.085 h-1 which was higher than reported for goats (3.97 ± 0.40 h-1) by [10]. On the other hand, it was lower than reported in normal chickens (6.95 ±0.47 h-1) by [16] and in buffalo calves (11.2 ± 0.42 h-1) by [6].

The distribution half- life ($t_{0.5\alpha}$) equal 0.137±0.002 h was lower than reported in normal chickens (13.52 minutes) by [8] and lower than recorded in goats (0.17 ± 0.03 h) by [10]. While it was higher than recorded in normal chickens (0.10 h) by [16] and in buffalo calves (0.06 ± 0.01 h) by [6].

The apparent volume of distribution at steady sate (Vdss) equal 1.76±0.014 L/kg was nearly equal to that recorded for broiler chickens (1.61±0.05 L/kg) by [16], (1.80±0.05 L/kg) by [17] and for goats (1.81 ± 0.60 L/kg) by [10]. On the other hand, it was lower than that recorded for broiler chickens (5.80 L/kg) by [8] and higher than that recorded for cats (0.97 ± 0.15 L/kg) by [11].

These variations might be attributed to species difference and healthy status as well as the method of analysis used [20]. This high Vdss value (1.76±0.014 L/kg) suggesting significant distribution and penetration of lincomycin into extravascular tissues. In this respect, [18] reported that lincosamides are widely distributed into most tissues including respiratory tissue, soft tissue, bones, and joints also tissue concentrations are higher than serum concentration.

The values of K_{12}, K_{21} are the first rate constants for the transfer of the drug from the central to peripheral compartment and from peripheral to central compartment respectively. In the present study, the values of K_{12} and K_{21} are 2.82±0.054 h^{-1} and 1.85±0.035 h^{-1} respectively, which indicated higher transfer of lincomycin from central to peripheral compartments. These values of K_{12} and K_{21} are in consistent of values obtained in broiler chickens (K_{12} =3.76 h-1, K_{21} = 1.86 h-1) by [16, 19], and (K_{12} =2.12 h^{-1}, K_{21} = 0.93 h^{-1}) by [17].

Lincomycin was eliminated after intravenous injection with elimination half-life ($t_{0.5\beta}$) equal 2.93 ± 0.014 h with the elimination rate constant (K_{10}) equal 0.651 ± 0.007 h^{-1}. This elimination half-life was nearly equal that recorded in pigs (2.8 h) by [7]. On contrast, it was higher than recorded in calves (2–2.5 h) by [5] and recorded in normal chickens (1.46 h) by [16]. While it was shorter than (3.56 ± 0.62 h), that reported in cats by [11] and (3.30 ± 0.08 h) in buffalo calves by [6].

The rate of total body clearance (CL tot) of lincomycin following a single intravenous injection was 0.457±0.004 L/h/kg. This value was equal to that reported in calves (0.486 L/h/kg) by [5] and in normal chickens (0.477±0.015 L/h/kg) by [17]. While it was lower than reported in goats (2.11 L/h/kg) by [10].

The area under the first moment curve (AUMC) of lincomycin following a single intravenous injection was 170.42 ± 2.236 mg /ml/h. This value was higher than that recorded in swine (27.2 ± 6.0 mg. h/ml), by [21], in goats (9.63 ± 1.12 mg /ml/h) by [10], in cats (98.47 ± 40.83 mg /ml/h) by [23], and in normal chickens (160.50 ± 14.55 mg. h/ml) by [17].

The mean residence time of lincomycin (MRT) was 3.87 ± 0.020 h. This value was close to that reported in swine (3.52 ± 0.11 h) by [22] and in normal chickens (3.775 ± 0.057 h) by [17]. On the other hand, this value was higher than that recorded in swine (2.6 ± 0.4 h) by [21] and higher than that recorded in goats (0.95 ± 0.25 h) by [10]. While it was lower than reported in cats (4.49 ± 1.11 h) by [11], and (5.50 ± 1.64 h) by [23] and in buffalo calves (4.32 ± 0.11 h) [6].

Qualitative and quantitative differences in dosage might be attributed to these differences in results. These variations in pharmacokinetic parameters were relatively common and frequently related to method used, healthy status of animal and specific interspecies variation [20].

In the present study, Lincomycin reached its maximum plasma concentration (7.08 ± 0.16 µg/ml), one-hour post administration and could be detected in plasma for 8 hours. This C_{max} was higher than recorded in chickens (1.62 µg/ml) by [8] and in pigs (5 µg/ml) by [21] and (5.15±0.18 µg/ml) by [22]. On the other hand, it was lower than reported in cat (22.52 ± 10.97 µg/ml) by [23] and in normal chickens (10.72 ± 0.22 µg/ml) by [17].

Lincomycin reached its maximum plasma concentration (C max = 7.08 ± 0.16 µg/ml), one-hour post administration and could be detected in plasma for 8 hours. This C max was higher than (1.62 µg/ml) recorded in chickens by [8] and in pigs (5 µg/ml) by [21] and (5.15±0.18 µg/ml) by [22]**.** On the other hand, it was lower than (22.52 ± 10.97 µg/ml) reported in cat by [23] and (10.72 ± 0.22 µg/ml) in normal chickens by [17].

The present results revealed that lincomycin reached its maximum plasma concentration after maximum time (t max) of 1.16 ± 0.02 h. This result was lower than reported in fasted pigs (2.9 ± 1.2 h) by [21]. While it was higher than that recorded in normal chickens (0.80 h) by [8], and (0.76 ±0.02 h) by [17] and in cats (0.80 ± 0.11h) by [11].

Lincomycin was rapidly absorbed and distributed after a single oral administration with distribution rate constant (α) = 2.24 ± 0.01 h-1 and distribution half- life ($t_{0.5\ \alpha}$) = 0.310 ± 0.01 h. Lincomycin was eliminated with the elimination half-life ($t_{0.5\ \alpha}$) of 3.35 ± 0.011 h. this result was in agree with that recorded in normal chickens (3.35 h) by [8]. While it was lower than that recorded in cats (4.12 ± 1.44 h) by [23], and it was higher than recorded in normal chickens (1.74±0.05 h) by [17].

Lincomycin administrated by oral route was rapidly absorbed from the gastrointestinal tract. The obtained results revealed that the calculated systemic oral bioavailability percent was 71.32 ± 2.62 %. These values

indicated rapid, excellent absorption of the drug after oral administration. Similar high oral bioavailability was reported in pigs (73%) by [21], in goats (91.72 ± 4.45%) by [10], in cats (81.78 %) by [11] and (80.13± 4.68%) in normal chickens by [17]. On contrast, this value was higher than recorded for normal chickens (28.88%) by [8].

The present result revealed that lincomycin could be detected in a therapeutic level for 8 hours in plasma following repeated oral administrations (1.62 ± 0.033 µg/mL). These concentrations exceeded the minimum inhibitory concentration value of lincomycin for Staphylococcus spp. (1.0 µg/mL) and for Streptococcus spp. (0.4 µg/mL) recorded by [18].

One of the aims of this study was to determine if there is a pharmacokinetical interaction between lincomycin and amprolium in broiler chickens. In this respect, the obtained plasma levels following repeated oral administration of lincomycin in chickens previously given amprolium (1.45 ± 0.042 µg/mL) were significally lower than those in normal chickens (1.62 ± 0.033 µg/mL). Thus, the concomitant administration of both drugs resulted in a lowered lincomycin concentration at different time intervals after dosing when compared with lincomycin alone.

This result was in agree with that recorded in chickens [24], as they recorded that the mean serum concentrations of levofloxacin were significantly lower in amprolium pretreated broilers compared to control broilers. Similar findings were previously reported for amprolium with enrofloxacin in broilers [25], who reported that, coccidia infected birds pretreated with amprolium exhibit a lower serum enrofloxacin concentration if compared with control birds. While these results are differ from another study in chickens by [26], who recorded that ampicillin concentration following repeated oral co-administrations with amprolium revealed a significant increase in serum drug concentration when compared with ampicillin alone.

The pharmacokinetic parameters of lincomycin during repeated oral administration in chickens previously given amprolium revealed that amprolium resulted in a significant increase in the distribution rate constant (3.92±0.145 h^{-1}) in chickens previously given amprolium, than in normal chickens (1.45±0.042 h^{-1}). The distribution half- life ($t_{0.5\ \alpha}$) was significantly decreased in chickens previously given amprolium. This result was in agree with that recorded in chickens by [27], as they recorded that diclazuril and halofuginone resulted in a significant short distribution half-life of doxycycline.

The maximal plasma concentration (C_{max}) of lincomycin was significantly lowered in chickens previously given amprolium than in normal chickens in all doses. Similar observation was previously reported in broiler chickens by [24] as they recorded that the mean serum concentrations of levofloxacin were significantly lower in amprolium-pretreated broilers when compared with control ones.

The time required for lincomycin to reach its maximum concentrations (t_{max}) showed non-significant decrease in all doses. The maximal plasma concentration (C_{max}) of lincomycin was significantly lowered in chickens previously given amprolium than in normal chickens in all doses. The elimination half- life ($t_{0.5(\beta)}$) of lincomycin was none significantly decreased in chickens previously given amprolium when compared with normal chickens. Lincomycin was cleared by all clearance processes (CL_{tot}) in the body with a lower significant rate than in normal chickens. The lower calculated C_{max} for lincomycin in chickens pretreated with amprolium (C_{max} = 7.29±0.204 µg/ml) compared with normal chickens (C_{max} = 8.13± 0.244 µg/ml) was associated with shorter ($t_{0.5\beta}$) of lincomycin in chickens pretreated with amprolium ($t_{0.5\beta}$) = 3.27±0.118 h) if compared with normal ones (($t_{0.5\beta}$) = 3.51±0.098h). This might be agreed with pharmacological interaction previously recorded for salinomycin in broiler chickens by [28], who found that serum concentration of amoxicillin (C_{max}) was significantly lower in salinomycin treated chickens. In the current study, the lower C_{max} of lincomycin in chickens pretreated with amprolium could be explained on the basis of the effect of amprolium on microsomal enzymes of liver. Similar observation was previously reported by [29], on co-administration of enrofloxacin with albendazole in goats. Both albendazole and toltrazuril are highly metabolized to sulphone in liver [30], a phenomenon explaining the probability of both drugs in inducing CYP 450 enzymes in animals and birds and consequently the rapid metabolism and lower C_{max} of lincomycin. Pretreatment of chickens with amprolium for five days before lincomycin administration is enough time to induce liver microsomal CYP 450 enzymes, although [29] found that a single dose of albendazole was sufficient to induce such induction in goats. While these results were differ from that of [26] in broiler chickens, who reported that the kinetic parameters of ampicillin following repeated oral co-administrations with amprolium revealed a significant increase when compared with ampicillin alone.

Tissue residues (ug/g) of lincomycin following repeated oral administration of twice daily for five consecutive days in normal chickens and in chickens previously given amprolium once daily for 5 consecutive days revealed wide distribution of the drug in the tested tissues, (brain, lung, heart, gizzard, liver, spleen, kidney, breast muscles, thigh muscles, fat and skin). In this respect, [18] reported that lincosamides are widely distributed into most tissues, including respiratory tissue, soft tissue, bones, and joints. High concentrations were found one day after the last dose in the liver, followed by kidney (2.13 ± 0.090, 1.90 ± 0.080 ug/g) respectively, and

the lowest concentrations were found in breast and thigh muscles. These results are in agreement with [17] who reported that highest concentrations of lincomycin were found in the liver followed by kidney (3.82 ± 0.63, 3.65 ± 0.71 ug/g) respectively. On contrast, [16] found that the highest concentrations were found after 0.25 hour in the kidney (28.5 ± 2.36 ug /g) followed by liver (25.83 ± 0.83 ug /g) after repeated intramuscular administration.

Lincomycin residues couldn't be detected by spectrophotometer assay in all assayed tissues after five days from the last dose in normal chickens and only after four days in chickens previously given amprolium. These findings may be attributed to the high clearance of lincomycin indicated the reduced possibility of finding residues a few days after treatment and the necessity of shorter withdrawal time for this antimicrobial.

These results were nearly consistent with those reported in pig by [7] as they recorded that lincomycin persisted in tissues up to 96 hours. Also, this result was in agreement with that recorded in broiler chickens by [17] as they recorded that lincomycin was still detected in all tested tissues up to 4 days after stopping its administration.

Amprolium resulted in a significant decrease in the tissue concentrations of lincomycin when compared with normal chickens. Similar findings were previously reported for broiler chickens by [24] as they mentioned that, lower tissues concentration of the drug at different time interval after stopping dosage regimen in amprolium-pretreated chickens as compared with values recorded in control birds. Also [17] found that tissues concentration of lincomycin were significantly lower than those in chickens fed on acidifiers free ration. On the other hand, these findings are different from that of [31] in broiler chickens, who found that the anticoccidials (diclazuril and halofuginone), prolonged the withdrawal time of the tested antibiotics (tylosin and doxycycline), and by [26], who recorded that amprolium resulted in a significant increase of ampicillin concentrations in serum and different tested tissues when compared with ampicillin alone.

The lower tissues concentration of lincomycin at different time intervals in chickens previously given amprolium as compared with values recorded in normal chickens in the present study could be attributed to the inducing effect of amprolium on liver microsomal enzymes.

CONCLUSION

It is concluded that administration of amprolium before lincomycin in broiler chickens would altered its kinetic profiles as well as tissue residues of oral lincomycin. Therefore, under this condition, the dose of lincomycin administration by oral route needs to be carefully adjusted.

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