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IMPACT OF TOXY-NIL AS ANTIMYCOTOXIN ON THE DISPOSITION KINETICS OF LINCOMYCIN IN BROILER CHICKENS

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SUMMARY

Pharmacokinetics of lincomycin was studied following single intravenous (I.V.) and oral administrations (20 mg. kg^{-1} b. wt) in both control and toxy-nil medicated chickens. Lincomycin plasma concentration was determined by microbiological assay method. Following I.V. injection, lincomycin plasma concentration versus time curve was best fitted a 2- compartment open model. Toxynil significantly decreased both the distribution and elimination half-lives of lincomycin from 0.28 ± 0.01 and 1.27 ± 0.06h in the control group to 0.19 ± 0.006 and 0.95 ± 0.04 h in toxy-nil medicated chickens, respectively. The volume of drug distribution at steady state (V_{dss}) and the rate of its total body clearance (CL_B) were significantly increased in toxy-nil medicated chickens (1.72 ± 0.08 $L.Kg^{-1}$ and 1.95 ± 0.07 $L.Kg^{-1}.h^{-1}$, respectively) as compared with that in the control ones (1.38 ± 0.05 $L.Kg^{-1}$ and 0.85 ± 0.03 $L.Kg^{-1}.h^{-1}$, respectively). Following oral administration, the absorption half-life ($t_{1/2\ ab}$) was significantly prolonged in toxy-nil medicated birds than in the control ones (0.22 ± 0.016 and 0.163 ± 0.013 h, respectively). This associated with a significant decrease in the drug peak plasma concentration (3.54 ± 0.24 µg. ml^{-1}) than in the control one (11.56 ± 0.75 µg.ml-1). The systemic bioavailability (F) was significantly decreased from 73.25 ± 5.08 % in the control group to 38.25 ± 2.89% in toxy-nil medicated one. In conclusion: concomitant administration of lincomycin and toxy-nil in broiler chickens should be hindered, as the interaction between both significantly reduces lincomycin oral absorption and enhance its elimination which consequently decreases its therapeutic efficacy.

INTRODUCTION

Antimycotoxins, mould inhibitors and antifungals

are important management tools that have been approved for using as a therapeutic and prophylactic protocol against mycotoxicosis which constitutes a major economic problem in poultry production allover the world. Toxy-nil is a commercial antifungal, mould inhibitor and anti-mycotoxin compound used either in drinking water or feed stuffs (jeresiunas and Triukas, 2000). It is formed of specially designed mixture of mould inhibitors and toxinbinders including citric (6%), phosphoric (6%), lactic (2%) and formic (0.2%) acids, propylenglycol (10%) and dried yeast (5%). These compounds increase the probability for occurrence of drug-drug interactions, so the kinetic and the therapeutic efficacy of any other co-administered drug will be consequently altered (Gillum et al., 1993). So, studying the pharmacokinetic interactions between these compounds and the different antibiotics that commonly used in poultry is of a great importance to optimize their therapeutic dosages on a scientific basis.

Lincomycin is one of lincosamide antibiotics isolated from *Streptomyces Lincolensis* (Mason et al., 1962 and Merck Index, 1976) that has a bacteriostatic effect against Gram +ve bacteria and *Mycoplasma Spp.* (Arnold and Ellis, 2002). It has a good therapeutic effect in treatment of many poultry diseases including CRD (Chaleva et al., 1994), necrotic entritis caused by *Clostridium perfringens* A & C (Hamdy et al., 1983) and early chick mortality caused by Staphylococcus aureus (Hamdy et al., 1980). Therefore the purpose of this study was to investigate the effect of toxy-nil as a commercial antimycotoxin on the disposition kinetics of lincomycin following its intravenous and oral administration in broiler chickens.

MATERIALS AND METHODS

Drugs

1- lincomycin hydrochloride : was obtained as a pure powder (100%) highly soluble in water, supplied from Jordan Vet. and Med. Ind. Co. (Jovet).

2- Toxy - Nil TM plus : was obtained in a liquid form . Produced by Nutri - AD International (Belgium).

Birds

Twenty-five healthy broiler chickens with average of 30-40 day and average body weight of 1.5-2 kg were used in this study. They were housed in cages, fed both antibacterial and antifungal free balanced ration for 15 days prior to starting of experiment, with free access of water.

Experimental design

Twenty chickens were classified into 2 equal groups (of 10 chickens each). Chickens in the 1st group were maintained for drinking non- medicated water (control group), while those in the 2nd one were allowed to drink toxy-nil containing water (0.2ml.L^{-1}) (toxy-nil medicated group) for 5 consecutive days. At the 5th day lincomycin was injected in a single intravenous dose (20mg. Kg-1b. wt) in the left brachial wing vein of each

chicken in both groups. Blood samples (2ml) were collected from the right brachial vein of each chicken by puncture method in a heparinized tubes just before injection and at 10, 20, 30, 45 min and 1, 2, 4, 6, 8 and 10 h post injection. Samples were immediately centrifuged at 3000 rpm for 5 minutes, then plasma was collected and stored at -20°C until assayed for lincomycin concentration.

Chickens in both groups were kept without any drug administration and allowed to drink non-medicated water for 15 days as a wash-out period. Then, chickens in the 1st group were left to drink non-medicated water, while those in the 2nd one offered toxy-nil medicated one in the same previous concentration for 5 censecutive days. At the 5th day, lincomycin was orally administrated in a dose of 20 mg.kg^{-1}b. wt. to chickens of both groups (food was withheld 6 hs before oral dosing unit 4 hs after drug administration). Blood samples were collected from the wing vein of each chicken into heparinized tubes at the same time intervals in the intravenous injection. All samples were immediately centrifuged, then plasma was collected and frozen at -20°C until assayed.

Analytical Procedure

Lincomycin plasma concentration was determined using the microbiological assay technique described by Arret at al., (1971) using *Sarcina lutea* (ATCC 9341) as the tested organism. Standard curve was constructed using antibacterial-free pooled plasma samples collected by slaughtering of the rest five non-medicated chickens. The lower detectable limit of licomycin assay was (0.16 µg. ml^{-1}). The *in vitro* protein binding percent of lincomycin was determined using the method of Craig and Suh (1980) with concentrations of 10, 5, 2.5, 1.25 and 0.625 ug.ml^{-1}.

Pharmacokinetic analysis

A computerized curve stripping software program (Rstrip, Micromath Scientific Software, Salt lake city, UT, USA) was used in the determination of the best-fit compartmental model and for estimation of the model dependent pharmacokinetic parameters. Following I.V. injection, Lincomycin plasma concentration time data for each chicken was fitted a two-compartment open model according to the following equation:

$$Cp = Ae^{-\alpha}+Be^{-\beta t}.$$

Where Cp is the drug concentration at time t, A and B are the intercepts of the distribution and elimination lines with the concentration axis, respectively, they were expressed in ug.ml^{-1}, α and β are the distribution and elimination rate constants, respectively,expressed in units of reciprocal time (h^{-1}) while e is the base of natural logarithm. The distribution and elimination half lives ($t_{1/2}\alpha$ and $t_{1/2\beta}$), the rate constants for drug

transferring from central compartment to peripheral one (K_{12}) and from tissues to central compartment again (K_{21}), the volume of distribution at steady state (V_{dss}) and the total body clearance (CL_B) were calculated according to standard equations (Baggot, 1978 and Gibalidi and Perrier, 1982) as follows:

$$t_{1/2}\,\alpha \text{ or } B = \frac{0.693}{\alpha \text{ or } B} \quad (h)$$

$$CL_B = K_{eL}.Vc \quad (L.h^{-1}.Kg^{-1})$$

$$V_{dss} = \frac{(K_{12}+K_{21})}{K_{21}} \quad Vc\ (L.kg)$$

Following oral administration, data were analyzed by compartmental and non-compartmental methods based on the statistical moment theory (Yamaoka et al., 1978). The peak plasma concentration (C_{max})and the time needed to reach the peak plasma concentration (T_{max}) were calculated mathematically by the following equations:

$$T_{max} = \frac{2.303}{Ka-Kel} \log \frac{K_a}{K_{el}}$$

$$C_{max} = Ae^{-Ka\ tmax} - Be^{-Kel\ tmax}$$

Where K_a is the absorption rate constant (h^{-1}) and Kel is the elimination rate constant (h^{-1}).

$AUC_{-\infty}$ is the area under the plasma concentration time cruve from zero to the infinity by the trapezoidal rule. The systemic bioavailability (F) was also calculated as $AUC_{oral}/ AUC_{i.v} X100$.

The obtained results are represented as mean ± standard error (S.E.). The pharmacokinetic parameters in presence and absence of toxy-nil were statistically analyzed using student's t-test (Snedecor and Cochran, 1976).

RESULTS

Semilogarithmic graph of the mean Lincomycin plasma concentrations versus time following single intravenous (i.v) and oral dosing (20 mg. Kg^{-1}b. wt.) in control normal and toxy-nil medicated chickens are shown in fig. 1 and 2, respectively. These are demonstrated that lincomycin concentrations were lowered in toxy-nil medicated chickens than in control ones at the same time intervals. The pharmacokinetic parameters of the tested drug following i.v. and oral administrations are recorded in tables 1 and 2, respectively. Following i.v. injection, the drug showed a distribution half-life ($t_{1/2\alpha}$) of 0.28± 0.01 and 0.19 ± 0.006h, and elimination half -life ($t_{1/2\beta}$) of 1.27±0.06 and 0.95±0.04h in control and toxy-nil medicated chickens , respectively. The volumes of drug distribution at the steady stae (Vdss) were 1.38±0.05 and 1.72±0.08 $L.Kg^{-1}$, and the volume of central compartment (Vc) was 1.04 ± 0.03 and 1.02 ± 0.04 $L.Kg^{-1}$ in the control group and toxynil medicated one, respectively. The drug was cleared from the body (CL_B) at a rate of 0.85

± 0.03 $L.h^{-1}. Kg^{-1}$ in the control chickens and 1.95±0.07 $L.h^{-1}. Kg^{-1}$ in toxy-nil medicated ones.

Following oral administration, Lincomycin achieved its peak plasma concentration (C_{max}) of 11.56 ± 0.75 and 3.54 ± 0.24 $\mu g.ml^{-1}$ at time (t_{max}) of 0.52 ± 0.03 and 0.63 ± 0.04 h in both control and toxy-nil medicated chickens, respectively. The systemic bioavailability was 73.25 ± 5.08% in control birds and 38.25 ± 2.89% in toxy-nil medicated ones.

The average value of protein binding percentage of lincomycin in chicken's serum was 29.14 ± 1.75%.

DISCUSSION

This present work was performed to investigate the effect of toxy-nil as a commercial antimycotoxin product on the pharmacokinetics of lincomycin following single i.v and oral administrations (20 mg. Kg^{-1} b.wt) in healthy broiler chickens. The obtained results demonstrated that concomitant administration of both drugs resulted in a lowered lincomycin plasma concentration at different time intervals after dosing, as compared with that administered alone. Following i.v. injection, lincomycin plasma concentration follows a 2- compartment open model in both control normal and toxy- nil medicated chickens. The drug was rapidly distributed when administered simultaneously with toxy- nil that evidenced by a short distribution half - life($t_{1/2\alpha}$) (0.19±0.006h) than when administered alone (0.28±0.01h). This was also confirmed by the highered values of both distribution rate constant from central compartment to the peripheral one (K_{12}) (0.79±0.04h^{-1}) and the ratio of distribution rate between compartments (k_{12} / k_{21}) (0.61 ± 0.03) in toxy-nil medicated chickens than in the control ones (0.54 ± 0.02 h^{-1} and 0.36 ± 0.02, respectively). Gilman et al., (1980) refared that Vdss value reflect the degree of drug distribution to the peripheral tissues. Accordingly toxynil induces a significant increase in lincomycin tissue distribution indicated by its elevated Vdss value in toxy-nil medicated chickens (1.72 ± 0.08 $l.kg^{-1}$) compared with the lowered one (1.38 ± 0.05 $l.kg^{-1}$) in the control normal chickens. The short elimination half-life ($t_{1/2\beta}$) observed in toxy-nil medicated birds (0.95±0.04h) than that in the control ones (1.27±0.06h) revealed rapid lincomycin elimination in presence of tox-ynil, which may be resulted from enhancement of lincomycin metabolism by the active constituents of toxy-nil. In this respect, Nishimaki et al., (1991) reported that sorbic acid, which is one constituent of the tested compound, has a moderate inducing effect for sorboyl-CoA reductase and 2.4 - dienoyl-CoA reductase enzymes in mouse liver. Propionate also was found to has a great enhancing effect on the hepatocyte metabolism (petitet et al., 1998), furthermore, yeast extract showed a highly stimulant ef-

fect on glucose metabolism in rat adipocytes (Edens et al., 2002). The rate of drug total body clearance (CL_B) was higher (1.95±0.07L.h^{-1}.kg-1) in toxy nil medicated group than in the control one (0.85±0.03L.h^{-1}.kg^{-1}). This is an expected result for its rapid elimination in presence of toxy-nil. In addition, the pH of the renal tubules and lower gut of toxy-nil medicated chickens might be shifted from the normal alkaline reaction (in normal chickens) to acidic one under the influence of its acidic constituents (propionic, sorbic and formic acids). Consequently, Lincomycin which is a basic drug with a pka value of 7.6 (Ziv and Sulman, 1973) and mainly excreted via the bile and the urine (Rang and Dale, 1991) will be more ionized in this acidic pH and so rapidly excreted (Harold and Walter, 1998). Following oral dosing, concomitant administration of lincomycin and toxy-nil resulted in a lowered lincomycin plasma concentration than when administered alone. Lincomycin was slowly absorbed in toxy-nil medicated chickens which revealed by a significant lowering in its absorption rate constant (Kab) (2.83 ±0.17h $^{-1}$) associated with significant prolongation in its absorption half - life (t $^{1}/_{2}$ ab) (0.22 ± 0.016 h) compared with that in control group (4.17 ± 0.26 h^{-1} and 0.163 ± 0.013 h). The peak lincomycin plasma concentration (Cmax) was greatly lower when administered in concomitant with toxy-nil(3.54 ± 0.24 µg. mL^{-1}) than when administered alone (11.56 ± 0.75 µg. mL^{-1}). However time taken to reach these peak concentrations was non significantly differed. These findings evidenced that toxy-nil may hinder the oral absorphon of lincomycin from chicken's gut .Oral absorption of any drug is controlled by the pH partition hypothesis (Hogben et al., (1959). According this theory, basic drugs are less absorbed from the more acidic contents in the gut (Baggot, 1977). So the lowring in Lincomycin absorption that concomitantly administered with toxy - nil might be correlated to the lowering in the pH of chicken's gut in toxy-nil medicated group to the acidic side, and so enhancing lincomycin ionization (which is a basic drug) and consequently decrease its absorption. In this respect, Dorrestein and Vanmiert, (1988) reported that oral medication in birds is greatly afected by the pH of the gut.

Concomitant administration of lincomycin and toxy-nil resulted also in a rapid drug elimination which observed by a significant short elimination half - life (t$^{1}/_{2}$el) in toxy - nil medicated birds (1.25±0.06h) compared with that in the control ones (1.72 ± 0.08 h). Each of sorbic acid, propionic acid and yeast extact which are main components of toxy-nil compound exhibit some metabolizing stimulant effects (Nishimaki et al., 1991, Petitet et al., 1998, and Edens et al., 2002, respectively), these may also enhance lincomycin metabolism. In the same time the acidic constituents of toxy-nil enhance lincomycin excretion via the bile and the urine as previously discussed follow-

ing the i.v. injection in this study. The systemic bioavailability of lincomycin was greatly reduced in presence of toxy-nil (38.25 ± 2.89 %) as compared when administered alone (73.25 ± 5.08%).

In conclusion, concomitant administration of lincomycin and toxy-nil should be hindered in broiler chickens, as the interaction between both decreases the oral absorption of lincomycin and enhances its elimination, which consequently reduces its therapeutic efficacy.

Table (1): Pharmacokinetic parameters of Lincomycin following a single intravenous injection (20 mg. kg^{-1}b.wt) in control normal and toxy-nil medicated chickens .(Mean ± S.E., n=10).

Parameter	Unite	Lincomycin	Lincomycin + toxy-nil
α	h^{-1}	2.25 ± 0.14	3.15 ± 0.18**
A	$\mu g.ml^{-1}$	8.34 ± 0.52	15.38 ± 0.97***
$t_{1/2\alpha}$	h	0.28 ± 0.01	0.19 ± 0.006***
β	h^{-1}	0.533 ± 0.03	0.78 ± 0.04**
B	$\mu g.ml^{-1}$	10.46 ± 0.75	4.56 ± 0.16***
$t_{1/2\beta}$	h	1.27 ± 0.06	0.95 ± 0.04**
K_{21}	h^{-1}	1.49 ± 0.08	1.35 ± 0.05
K_{el}	h^{-1}	0.94 ± 0.04	1.68 ± 0.08***
K_{21}	h^{-1}	0.54 ± 0.02	0.79 ± 0.04***
K_{12}/K_{21}		0.36 ± 0.02	0.61 ± 0.03***
Vc	$L.Kg^{-1}$	1.04 ± 0.03	1.02 ± 0.04
V_{dss}	$L.Kg^{-1}$	1.38 ± 0.05	1.72 ± 0.08**
AUC	$\mu g.ml^{-1}h^{-1}$	22.17 ± 1.68	10.63 ± 1.04***
CL_B	$L.Kg^{-1}h^{-1}$	0.85 ± 0.03	1.95 ± 0.07***

** Significant at P≥0.01
***Significant at P≥0.001

Table (2): Pharmacokinetic parameters of Lincomycin following a single oral administration (20 mg. kg^{-1}b.wt) in control normal and toxy-nil medicated chickens .(Mean ± S.E., n=10).

Parameter	Unite	Lincomycin	Lincomycin + toxy-nil
Kab	h^{-1}	4.17 ± 0.26	2.83 ± 0.17**
A	$\mu g.ml^{-1}$	15.36 ± 0.94	5.61 ± 0.35***
$t_{1/2ab}$	h	0.163 ± 0.013	0.22 ± 0.016*
K_{el}	h^{-1}	0.41 ± 0.03	0.56 ± 0.04*
B	$\mu g.ml^{-1}$	8.05 ± 0.52	3.08 ± 0.21***
$t_{1/2el}$	h	1.72 ± 0.08	1.25 ± 0.06**
T_{max}	h	0.52 ± 0.03	0.63 ± 0.04
C_{max}	$\mu g.ml^{-1}$	11.56 ± 0.75	3.54 ± 0.24***
AUC	$\mu g.ml^{-1}h^{-1}$	17.40 ± 1.39	4.16 ± 0.27***
F	%	73.25 ± 5.08	38.25 ± 2.89***

* Significant at P≥0.05
** Significant at P≥0.01
*** Significant at ≥ 0.001

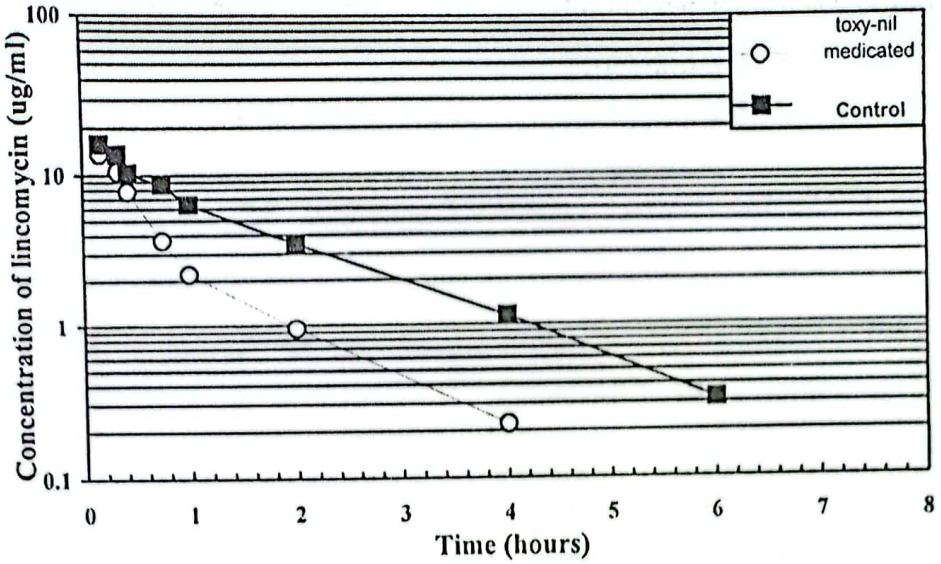

Fig.(1): Semilogrithmic graph depicting lincomycin plasma concentration-time course after a single intravenous injection of 20mg/kg b.wt. in control normal and toxy-nil medicated broiler chickens.

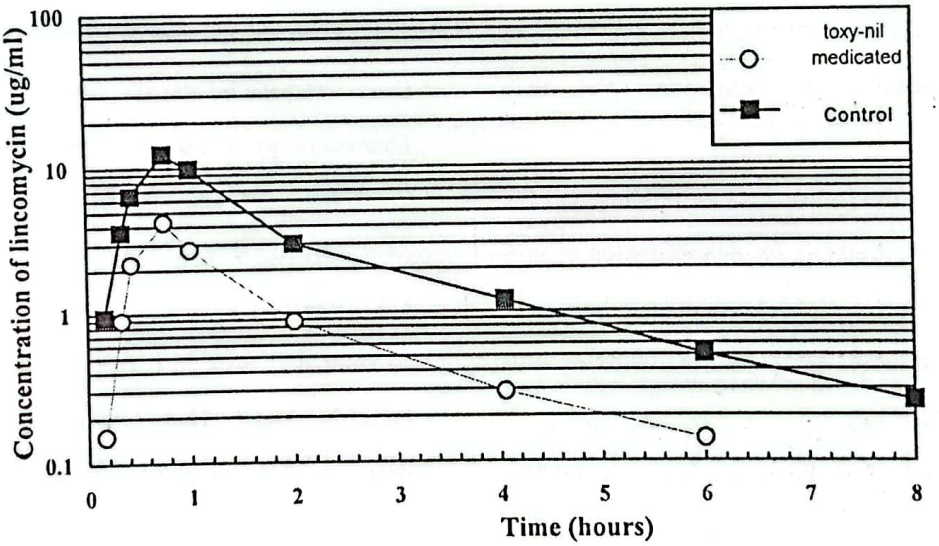

Fig.(2): Semilogrithmic graph depicting lincomycin plasma concentration-time course after a single oral dose 20mg/kg b.wt. in control normal and toxy-nil medicated broiler chickens.

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