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Pharmacokinetics of Tildipirosin in Healthy and *Mycoplasma gallisepticum* Infected Chickens

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ABSTRACT

The pharmacokinetic features of tildipirosin were explored following a single dose of 4 mg/kg in healthy and *Mycoplasma gallisepticum* (*M. gallisepticum*) infected chickens. Eighteen healthy chickens (400-500g) were allocated into 3 groups (n=6); group I and II were received tildipirosin by intravenous (IV) and intramuscular (IM) routes, respectively. Group III was experimentally infected with *M. gallisepticum* and injected IM with tildipirosin after confirming the infection (9-10 days after inoculation of *M. gallisepticum).* Plasma samples were harvested at different time points until 14 days after tildipirosin injection to measure its concentrations using HPLC. After IM administration of tildipirosin in healthy chickens, the maximum concentration in plasma (C_{max}), time to achieve C_{max} (T_{max}), area under the plasma concentration time curve from 0 to last time ($AUC_{0\text{-}last}$), clearance (Cl-F-obs) and the absolute bioavailability were recorded to be 403.76ng/ml, 0.25 hr, 6.82 µg.hr/ml, 0.56L/hr/kg, and 103.50% respectively. C_{max} and $AUC_{0\text{-}last,}$ were significantly lower in infected than healthy chickens, while Cl-F-obs was significantly higher in infected than healthy chickens. Therefore, *M. gallispeticum* infection produced significant changes in some of the pharmacokinetic parameters of tildipirosin in chickens. Further studies are warranted to assess pharmacokinetic/ pharmacodynamic profile of tildipirosin against *M. gallisepticum* in chickens and to gain deeper insight into its safety utilization in chickens.

Key words: Chickens, HPLC, *Mycoplasma gallisepticum*, Pharmacokinetics, Tildipirosin.

INTRODUCTION

Tildipirosin, a semisynthetic macrolide antibiotic, has been marketed solely for veterinary use and is predominantly employed for management of respiratory diseases in bovine and pigs induced by multiple pathogens including *Actinobacillus pleuropneumoniae*, *Mannhemia haemolytica*, *Pasteurella multocida*, *Haemophilus parasuis* (EMA 2010; Torres et al. 2016). It is distinguished by its prolonged action after a single- dose injection regimen which subsequently reduce stress from frequent animal handling (Xiong et al. 2020). Tildipirosin's bacteriostatic effect is owed to its blocking action on the 50S ribosomal subunit of bacteria, hence hindering the protein production (Schlünzen et al. 2001).

The pharmacokinetics of tildipirosin have been explored in rabbits (Xiong et al. 2020), pigs (Rose et al. 2013), ewes (Galecio et al. 2020), goats (Elazab and Badawy 2020), dog (Wang et al. 2018), and cattle (Menge et al. 2012). Rapid absorption, massive distribution in to tissues, and prolonged elimination half-life are the prominent characteristics of tildipirosin in the aforementioned animal species. However, so far as the authors know, no literatures are available regarding the pharmacokinetics of tildipirosin in chickens.

Mycoplasma gallisepticum (M. gallisepticum) is the causative agent of chronic respiratory disease (CRD) in poultry. Tracheal rales, sneezing, nasal and ocular discharges are the main manifestations of *M. gallisepticum* infection in chickens (Levisohn and Kleven 2000). This infectious disease spreads by both horizontal and vertical routes between the avian population, causing decrease in weight gain, feed conversion, and increase in mortality rate with subsequent higher economic losses in poultry farms (Kleven 1990; Ley 2003). Antimicrobials play a pivotal role in controlling this infection (Zhang et al. 2016; 2018).

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The most frequently used antibiotics for treatment of *M. gallisepticum* infection are the macrolides, pleuromutilins, and tetracyclines (Gautier-Bouchardon et al. 2002). It is crucial to investigate the pharmacokinetics of these drugs in infected chickens as the pharmacokinetic features of drugs may be changed by pathophysiological alterations induced by infection (Huang et al. 2003).

Elucidating the pharmacokinetic profile of tildipirosin in *M. gallisepticum*- infected chickens may pave the way for conducting of future researches to study the possible utilization of tildipirosin in the treatment of CRD in chickens as it is imperative to search for novel effective antibiotics to combat resistant microorganisms. Thus, the objectives of the present work were: (a) to assess the disposition kinetics of tildipirosin in healthy chickens after intramuscular (IM) and intravenous (IV) administrations, and (b) to evaluate the influence of M. gallisepticum infection on the pharmacokinetics of tildipirosin in chickens.

MATERIALS AND METHODS

Materials

The antibiotic utilized in this research, tildiprosin solution (Zuprevo™, 18%), was manufactured by Intervet International Gmbh, MSD Animal Health, Germany. The standard of tildipirosin was supplied from Intervet International Company, Egypt. Formic acid, acetonitrile, diethyl ether, and dipotassium hydrogen phosphate (K2HPO4) were bought from Merck, Germany. All the employed chemicals were of HPLC-grade. *M. gallisepticum* field isolates (Eis3-C-10) GenBank accession No. (HQ 591355) was provided from Animal Health Research Institute, Egypt.

Birds

Eighteen healthy chickens (half male and female, 14 days of age, body weight of 400-500 gm) were purchased from Faculty of Agriculture, Mansoura University, Egypt. Chickens were evaluated to be healthy depending on physical examination. Also, PCR test of tracheal swabs was used to verify the absence of *M. gallisepticum* infection. Chickens were placed under convenient conditions (21-25°C, 45-70% humidity). They were acclimatized for 7 days prior to the study. They received drug-free diet with free access to water. All steps incorporating chickens were endorsed by Research Ethics committee of the Faculty of Veterinary Medicine, Mansoura University, Egypt (Approval No. R/45).

Experimental Design

Chickens were assigned randomly in to 3 groups (n=6, male: female, 1:1). Group I and II were received one dose of tildipirosin at 4mg/kg (Xiong et al. 2020) by IV and IM routes, respectively. Tildipirosin was injected into the right-wing vein and thigh muscle. Group III was inoculated by intranasal and intratracheal routes each with 0.1 ml containing 10^7CFU of *M. gallisepticum* culture (Gharaibeh and Hailat 2011). After the inoculation, the clinical signs were checked. The infection was emphasized by the appearance of the following manifestations: sinusitis, sneezing, conjunctivitis. Moreover, molecular identification of *M. gallisepticum* in tracheal swabs by PCR were conducted to verify the infection. After appearing of the symptoms and confirming the infection (9-10 days following *M. gallisepticum* inoculation), chickens in group III were injected intramuscularly with single dose of tildipirosin (4mg/kg). Blood samples (not >1% of bodyweight) were harvested from the left-wing vein of each chicken in EDTA tubes at time 0 (prior to tildipirosin injection), 5, 15, 30min,1, 2, 4, 8, 12hr, 1, 3, 4, 6, 8, 10, 12, and 14days after drug administration. Plasma was separated and preserved at -20°C until being investigated.

Analysis of Tildipirosin in Plasma Samples

Extraction of tildipirosin from Plasma Samples

Plasma samples were prepared based on previously reported technique (Lei et al. 2018). In brief, 200µl of dipotassium hydrogen phosphate was added to 500µl of plasma sample and then diethyl ether was added for extraction. The mixture was centrifuged and the supernatant was evaporated. Then, 0.1ml acetonitrile was used to redissolve the residue.

Chromatographic Conditions

The levels of tildipirosin in plasma were assessed utilizing a former published HPLC method (Lei et al. 2018; Elazab and Badawy 2020). The HPLC Agilent Series 1200 quaternary gradient pump, Series 1200 autosampler, Series 1200 UV VIS detector set at 289 nm, and HPLC 2D chemstation software (Hewlett-Packard, Les Ulis, France) were employed. Chromatographic separation was performed utilizing phenomenex C18 (5 µm, 250 mm x 4.6 mm). 0.15% formic acid in acetonitrile were the constituents of the mobile phase. The flow rate was 1.2 ml/min. The retention time was 3.3 min. Revalidation of the HPLC technique was carried out as revealed by European Medicines Agency protocol (Anonymous 2009) using chicken plasma (Table 1). A linear correlation was noticed in the standard curve in the range of 3.3-1000ng/ml.

Pharmacokinetic Analysis

Non- compartmental model [WinNonlin 8.3 software (Certara, USA)] was used in this study to estimate the pharmacokinetic parameters of tildipirosin for each chicken as mentioned by (Lei et al. 2018; Wang et al. 2018). Following IM administration, the maximum concentration in plasma (C_{max}) and the associated peak time (T_{max}) were visualized from the individual plasma concentration time curves. The linear trapezoidal method was employed to calculate the area under the plasma concentration- time curve ($AUC_{0-\infty}$) after IV injection. While, The $AUC_{0-\infty}$ was calculated using linear up-log down trepazoidal method following IM administration. The clearance (Cl-obs) was measured as Cl-obs=dose/AUC. Moreover, Vz-obs, apparent volume of distribution was determined using this equation: Vz=dose/ (λz X AUC). Bioavailability (F) was estimated by the following equation: $F=(AUC_{ev}/ AUC_{iv}) X100$. The elimination half-life ($T_{1/2}\lambda z$) was determined as $T_{1/2}\lambda z = 0.693/\lambda z$.

Statistical Analysis

Pharmacokinetics parameters are shown as geometric mean and range. T_{max} is presented as median and range. Wilcoxon's rank sum test was used to compare the pharmacokinetic parameters after IV and IM injections in

healthy chickens. Also it was used to compare the pharmacokinetic parameters between healthy and *M. gallisepticum* infected chickens after tildipirosin IM injection. Differences in the tildipirosin plasma concentrations between healthy and infected chickens were investigated employing repeated measure analysis of variance. $P<0.05$ was referred as statistically significant. This statistical analysis was conducted utilizing Prism 7.0 (Graph Pad, USA).

RESULTS

Chickens in group III, inoculated by *M. gallisepticum*, showed sinusitis, sneezing, conjunctivitis at 9-10 days of inoculation. Also, The DNA of *M. gallisepticum* was identified from the tracheal swab cultures obtained from all inoculated chickens at 10 days of inoculation. All chickens tolerate tildipirosin injection without exhibiting adverse reactions throughout the study.

Table 1. showed the validation parameters of the HPLC method utilized for measuring tildipirosin concentrations in plasma samples. The limit of detection and quantification of tildipirosin were 1 and 3.3ng/ml.

Fig. 1 and Fig. 2 represent the semilogarithmic plots of the mean plasma concentrations of tildipirosin at various time points after single IV and IM injections at 4mg/kg in healthy and experimentally challenged chickens with *M. gallisepticum*. Quantifiable concentrations of tildipirosin were still reported up to 10 and 12 days after IV and IM administration in healthy chickens, respectively. Meanwhile, after IM injection of tildipirosin (4mg/kg) in *M. gallisepticum* infected chicken, it could be quantified in plasma up to 10 days post administration. In addition, the plasma concentrations in infected chickens were lower at all-time points than healthy ones injected with tildipirosin intramuscularly. Significant differences were noticed at 15 min ($P<0.001$) and 30 min ($P<0.01$) following injection.

The pharmacokinetic parameters of tildipirosin of all experimental groups are listed in Table 2. No significant difference was found in the pharmacokinetic parameters between healthy chickens administered tildipirosin IM and those injected with it IV. Meanwhile, a marked decline ($P<0.05$) in the values of Cmax, $AUC_{0\text{-last}}$, $AUC_{0\text{-}\infty}$ were observed in *M. gallisepticum* infected chicken, injected with tildipirosin intramuscularly, when compared with the healthy ones in group II. Whereas, the value of Cl-F-obs was significantly higher ($P<0.05$) in infected than healthy chickens.

DISCUSSION

This is the first research about the pharmacokinetics of tildipirosin in chickens. The pharmacokinetic profile in this work elucidated that the C_{max} was 403.76µg/ml after IM injection in healthy chickens. This value was lower than that recorded in dogs (1051ng/ml) (Wang et al. 2018), swine (1000ng/ml) (Lei et al. 2018) rabbits (836.2ng/ml) (Xiong et al. 2020), and ewes (1264.4ng/ml) (Galecio et al. 2020) administered tildipirosin IM at 4 mg/kg, and in cattle (711ng/ml) (Menge et al. 2012) injected with tildipirosin SC at the same dose. These variations may be attributed to species difference. This recorded low plasma concentration may be

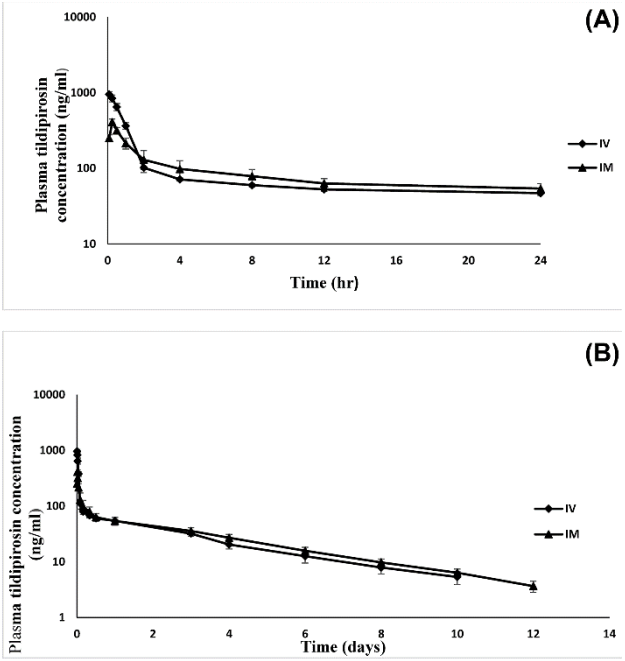

Fig. 1: Semilogarithmic plot of tildipirosin plasma concentrations in healthy chickens after IV and IM administrations at 4 mg/kg. Values are shown as mean ± SEM (n=6). (A) Elucidating the concentration-time within 24 hr. (B) Revealing the concentration-time during the whole process.

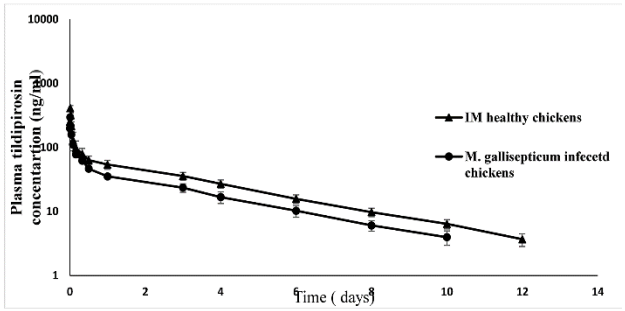

Fig. 2: Semilogarithmic plot of tildipirosin plasma concentrations after its IM administration (4mg/kg) in healthy and *M. gallisepticum* infected chickens. Values are expressed as mean ± SEM (n=6).

due to its high volume of distribution (55.73L/kg), as tildipirosin is characterized by its wide partitioning in to tissues owing to its lipophilic feature (EMA 2011). Former reports have revealed that tildipirosin concentrations were 159 times higher in bovine lungs compared to plasma concentration (Menge et al. 2012). Moreover, Rose et al. (2013) demonstrated that the level of tildipirosin in swine bronchial fluid was 681 times higher than that measured in plasma.

The present study showed that this drug was quickly absorbed following IM administration in healthy chickens as the time required to attain C_{max} (T_{max}) was short (0.25 hr). This value was similar to that previously published for rabbits (0.33 hr) (Xiong et al. 2020). In contrast, it was shorter than that reported for goats (0.5 hr) (Elazab and Badawy 2020), and ewes (0.62) (Galecio et al. 2020). This discrepancy may be attributed to the variations in the blood supply at the injection site between the species (Xiong et al. 2020).

Table 1: Validation parameters of the HPLC method used for analysis of plasma samples. Data for recovery are presented as mean±SEM.

Matrix	Average recovery (%)	Intra-day RSD (%)	Inter-day RSD (%)	LOD (ng/ml)	LOQ (ng/ml)
Plasma	97.21 ±1.56	2.59	2.90	1	3.3

Intra-day RSD and Inter-day RSD % (n=6, 25 ng/ml): Average recovery % (utilizing spiked concentrations (5-1000ng/ml) in triplicate investigation).

Table 2: Pharmacokinetic parameters of tildipirosin after its IV and IM administration at 4mg/kg in healthy and *M. gallisepticum* infected chickens

Parameters	Tildipirosin IV in healthy chickens	Tildipirosin IM in healthy chickens	Tildipirosin IM in *M.gallisepticum* infected chickens
C_{max} (ng/ml)	NA	403.76 (339-520)	293.41 (245-349)*
T_{max} (hr)	NA	0.25 (0.25-0.25)	0.25 (0.25-0.25)
λz (1/hr)	0.011 (0.010-0.013)	0.010 (0.009-0.011)	0.012 (0.008-0.014)
$T_{1/2}$ λz (hr)	60.61 (52.47-67.84)	66.06 (61.69-73.49)	58.55 (50.73-79.57)
$AUC_{0\text{-last}}$ (µg*hr/ml)	6.51 (5.52-8.74)	6.82 (4.54-10.65)	4.54 (3.15-5.56) *
$AUC_{0\text{-}\infty}$ (µg*hr/ml)	6.96 (5.98-9.62)	6.97 (4.86-10.95)	4.85 (3.40-5.83)*
Vz_obs (L/kg)	50.19 (40.69-62.33)	----------	----------
Vz_F_obs (L/kg)	----------	53.05 (32.5-75.17)	59.58 (46.61-94.78)
Cl_obs (L/hr/kg)	0.57 (0.42-0.67)	----------	----------
Cl_F_obs (L/hr/kg)	----------	0.56 (0.36-0.82)	0.82 (0.68-1.17)*
MRT (hr)	55.37 (43.54-73.73)	72.49 (66.21-78.52)	60.26 (46.61-72.93)
AUMCINF (hr*hr*µg/ml)	502.18 (373.16 -942.01)	627.76 (407.07-867.04)	378.14 (213.09- 513.48)*
F (%)	----------	103.50 (78.93-161.63)	--------

C max : Peak plasma; Tmax : Time to peak concentration; λz: , the first order rate constant; T1/2λz: elimination half-life; AUC0- last: area under the plasma concentration vs. time curve from 0 to last time; AUC0-∞: Area under the plasma concentration-time curve from 0 to infinite; Vz_obs: apparent volume of distribution in terminal phase; Vz_F_obs: volume of distribution per fraction of dose absorbed; Cl_obs: total body clearance; Cl_F_obs : The clearance per fraction of the dose absorbed; MRT: Mean residence time curve; AUMC: Area under the first moment curve; F mean systemic bioavailability. Data are presented as geometric mean and range (n=6). Tmax is shown as median and range. *P<0.05.

After IV and IM injection of tildipirosin in healthy chickens, the AUC0-last (6.96 and 6.82, respectively) were comparatively slightly higher than those registered for dogs (Wang et al. 2018), and rabbits (Xiong et al., 2020). On the other hand, they were less than those evaluated in cattle (Menge et al. 2012), and ewes (Galecio et al. 2020). The high absolute bioavailability calculated after IM injection (103.50%) suggesting high absorption rate of tildipirosin in chickens. This result was relatively similar to that of Wang et al. (2018) and Xiong et al. (2020) who reported that the absolute bio availabilities of tildipirosin were 112% and 105.4% in dogs and rabbits, respectively. On the contrary, the bioavailability estimated in the current study was higher than that calculated for pigs (85.5%) (Lei et al. 2018), goats (96.4%) (Elazab and Badawy 2020), and ewes (79.17%) (Galecio et al. 2020). This difference may be attributed to species variations.

The $T_{1/2}$λz reported in chickens was shorter than that declared in other species (cattle, pigs, dogs, goats, rabbits, sheep, and ewes) (Menge et al. 2012; Rose et al. 2013; Elazab and Badawy 2020; Xiong et al. 2020; Galecio et al. 2020). The plasma clearance of tildipirosin in healthy chickens (0.58L/hr/kg) was comparable to that revealed by Wang et al. (2018) in dogs (0.72L/hr/Kg), but higher than that recorded in goats (0.216L/hr/kg), and rabbits (0.23L/hr/kg) (Xiong et al. 2020). These dissimilarities may be attributed to species variations as avian species are known to have higher metabolic rate than mammals (Clarke et al. 2010).

As disease state has been associated with alterations of pharmacokinetic behavior of antibiotics (Baggot 1980), it is crucial to study the pharmacokinetic features of these drugs in diseased animals to adjust the appropriate dosage schedule for favorable clinical outcomes. In the present study, the pharmacokinetic parameters of tildipirosin were calculated in *M. gallisepticum* infected chickens after IM administration only as this route of delivery is easier to perform than IV injection in clinical practice. The findings of the current study revealed that *M. gallisepticum* infection statistically significant reduced the plasma concentrations of tildipirosin at 15 min, and 30 min after administration versus to healthy chickens. These lower plasma concentrations in infected chickens is likely due to the extensive distribution of tildipirosin to the diseased tissues (Baggot 1980; Riviere 2009). These findings were in concordance with those of Salman et al. (2016) who reported that the serum concentrations of tylvalosin was significantly lower in *M. gallisepticum* infected chickens compared to healthy ones. Similarly, Attia et al. (2018) mentioned that the serum levels of tilmicosin were markedly higher in healthy chickens than in *M. gallisepticum* and *Escherichia coli* infected ones.

Furthermore, a significant decrease in the values of Cmax, $AUC_{0\text{-last}}$, and $AUC_{0\text{-}\infty}$ were noticed in *M. gallisepticum* infected chickens when compared to the uninfected ones in group II. This indicates a slower absorption rate of tildipirosin injected intramuscularly in infected chickens versus the healthy ones. While, compared to healthy chickens, the infected group showed a faster clearance rate of tildipirosin. These results were consistent with those of Salman et al. (2016) who recorded a slower absorption rate and higher clearance rate of tylvalosin in *M. gallisepticum* infected chickens.

In conclusion, tildipirosin exhibited favorable pharmacokinetic properties in chickens with prompt absorption, high bioavailability, extensive distribution, and slow elimination after IM administration. Moreover, this study revealed that *M. gallispeticum* infection caused

significant alterations in some of the pharmacokinetic parameters of tildipirosin in chickens. Future studies are warranted to measure concentrations of tildipirosin in lung tissue and to study its MIC value and PK/PD profile against *M. gallisepticum* in chickens and to gain deeper insight into its safety utilization in chickens.

Author's Contribution

SE designed the study and wrote the manuscript. SE and YH conducted the animal experiment. NE performed the HPLC analysis. AA analyzed the data of this work and. All authors have read and approved the final manuscript.

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