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Pharmacokinetic Disposition and Residue Status of Tilmicosin in Broiler Chicken after 'In-Crop' and 'In-Water' Administration

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ABSTRACT

Background: The aim is to study the pharmacokinetics and tissue residue status of tilmicosin after administration through 'in-crop' and 'in-water' routes and compare the effectiveness of the two routes and establish a suitable dosage regimen for treating *Mycoplasma gallisepticum* infection in broiler chicken.

Methods: The plasma pharmacokinetic disposition of tilmicosin in broiler chicken was investigated after administration orally by direct deposition at crop (25 mg/kg body weight) or drinking water (40 mg/kg b.wt.). Residues of tilmicosin in tissues of broiler chicken were assayed. The plasma and tissue concentrations of tilmicosin were analyzed by reverse-phase high-performance liquid chromatography (HPLC) method. The plasma concentration-time data was described by the non-compartmental model for both routes and pharmacokinetic parameters were calculated. The data were statistically analyzed by Mann-Whitney U test

Conclusion: The mean plasma concentrations of tilmicosin in two routes tested (in-crop, in-water) were effective above MIC reported for *Mycoplasma gallisepticum* (0.05 µg/ml) up to 24 h. In addition, the drug residue in lungs was found at desirable concentration up to 22 days. However, residues of tilmicosin in tissues were above the advocated maximum residue limit (MRL) till 18th day in muscle and liver and till 22nd day in kidney. The results of the study indicate that the antimycoplasmal drug tilmicosin can be therapeutically efficacious after administration in crop as well as in drinking water.

Key words: Broiler chicken, Mycoplasmosis, Pharmacokinetics, Tilmicosin, Tissue residue.

INTRODUCTION

Poultry sector is increasingly contributing to the Indian economy during the last three decades (Mehta *et al*., 2016). Integrated broiler production is mainly concentrated in Andhra Pradesh, Tamil Nadu, Maharashtra, Karnataka and West Bengal contributing to nearly 85% of the total national production (Kalamkar, 2012). The negative impact on the Indian economy due to disease outbreak and reduced growth in commercial broiler industry is being averted by using prophylactic antibiotics and growth promoters (Kusiluka *et al*., 2005; Mariki, 2007).

Chronic respiratory disease (CRD) caused by *Mycoplasma gallisepticum* in broiler chicken causes major economic losses due to its high mortality rate, reduced productivity and down-gradation of carcass at slaughter (Feizi *et al*., 2013). The antimicrobial agents such as macrolides, pleuromutilins, tetracyclines and fluoroquinolones possess *in vitro* anti-mycoplasmal activity (Bradbury *et al*., 1994; Hannan *et al.,* 1997). Tilmicosin, a semi-synthetic macrolide derived from tylosin, is an approved and commonly used prophylactic and therapeutic anti-mycoplasmal agent in broiler industry (Taha *et al*., 1991). With a low 'minimum inhibitory concentration' (MIC), prolonged antibacterial activity and extensive tissue penetration (Abd El-Ghany, 2009; Jordan *et al*., 1993; Cherlet *et al*., 2002), tilmicosin is an effective anti-mycoplasmal drug.

Studies on the pharmacokinetics of tilmicosin administered directly into the crop in the broiler chicken have been done (Keles *et al*., 2001; Abu-basha *et al*., 2007; Rassouli *et al*., 2016). Yet, there is lack of authentic documentation on pharmacokinetics of tilmicosin administered *via* drinking water which is the general practice in large scale commercial broiler farms. Thus, the aim is to study the pharmacokinetics and tissue residue status of tilmicosin after administration through 'in-crop' and 'in-water' and compare the effectiveness of these two routes of administration.

MATERIALS AND METHODS

The analytical standard Tilmicosin was procured from Sigma Aldrich private limited, India. Tilmovet® 25% oral solution was

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obtained *gratis* from M/s HuvePharma Private Limited, Pune, India. Acetonitrile and Methanol (HPLC grade) were procured from Merck Specialities Private Limited, Mumbai. Ammonium Formate, Trifluoroacetic acid and Perchloric acid were from Himedia Labs Private Limited, Mumbai.

Thirty-seven healthy Vencobb broiler chicken, four to five weeks old, weighing 1.2 ± 0.2 kg were reared in a deep litter system with *ad libitum* water and poultry finisher mash (antibiotic and anticoccidial free). After one week of acclimatization period, the study was conducted with overnight fasting of birds before drug administration. The experimental design and bird usage was approved by Institutional Animal Ethical committee, Madras Veterinary College, Chennai, India (Approval Lr.no.2345/21/DFBS/IAEC/2016, dated: 26.10.2016).

Experimental design

The present experiment was conducted at Madras Veterinary College, Chennai, Tamil nadu during 2017. Initially, a pilot study was performed in six birds to choose the appropriate dose of tilmicosin for administration through drinking water. In the pilot study, three doses of tilmicosin *viz.* 25, 30 and 40 mg/kg b. wt (two birds for each dose) were mixed with drinking water for one day. The blood samples were collected at 0, 0.08, 0.25, 0.5, 1, 2, 3, 4, 8, 12, 24, 48 h post dosing. The plasma was separated and the plasma drug concentration was analyzed by HPLC. Based on the higher Cmax value, the dose of 40 mg/kg was fixed for the main study.

The main study was conducted in two phases. In phase I, the pharmacokinetic study was conducted in which 16 birds were randomly categorized into 'in-crop' and 'in-water' groups (8 birds/group). The dose rate of tilmicosin was 25 mg/kg b.wt (Jordan and Horrocks, 1996; Kempf *et al*., 1997) for 'in-crop' route and 40 mg/kg b. wt. (based on pilot study) for 'in-water' route. The blood samples were collected from median tarso-metatarsal vein at 0, 0.08, 0.25, 0.5, 1, 2, 3, 4, 8, 12, 24 and 48 h after drug administration. The plasma was separated by centrifugation at ~1000 x g for 10 min and stored at -20°C until analysis.

In phase II, residue study was conducted in 15 birds by administering tilmicosin through drinking water at the dose rate of 40 mg/kg b. wt for 5 days (as per therapeutic regimen). The birds were sacrificed on days 10, 14, 18, 22 and 25 post-last dose (three birds per day) and tissues such as lungs, liver, breast muscle, thigh muscle and kidney were collected, homogenized and stored at -20°C until analysis to assess the tissue residues.

Analytical method

The isocratic separation and quantification of tilmicosin was performed using Syncronis C18 column (5μm, 4.6 x 250 mm, Thermo-scientific, USA) in the UFLC Shimadzu system (Japan) which consisted of degassing unit, pump (Prominence, LC 20 AD), Photo Diode Array detector (Prominence, SPD-M20A), auto-sampler (Prominence, SIL-20 AC HT) and column oven (CTO-10AS VP). The mobile phase consisting of 0.1M ammonium formate: acetonitrile: methanol (60:30:10 v/v/v, pH adjusted to 5.0 with trifluoroacetic acid) was used at the flow rate of 1.2 mL/min and the detection wavelength was 287 nm (Keles *et al*., 2001). The column temperature was maintained at 30°C.

The plasma and homogenized tissue samples were extracted by liquid-liquid extraction. 50µl of perchloric acid (10%) was added to 450 µl of sample, vortexed for 30 seconds and centrifuged at 1000 x g for 5 min (Abu-basha *et al*., 2007). 10 µl of clear supernatant filtered through 0.2 µ HNN membrane was injected in HPLC system for analysis.

Drug-free poultry plasma and tissue homogenates were spiked with tilmicosin standards ranging from 0.01 to 5 µg/ml and the calibration curve was constructed. The HPLC method was validated for tilmicosin quantification in chicken plasma and tissues. The calibration curves were linear over a range of 0.05 - 5µg/ml with r^2 value of 0.999. The inter- and intra-day precision were 13.84% and 4.85% respectively. The limit of quantification was 0.06 µg/ml and the analytical recovery was 100.66 %. Hence, the HPLC method in this study was highly sensitive and precise with excellent reproducibility.

Pharmacokinetic analysis

The pharmacokinetic parameters such as area under curve (AUC), area under moment curve (AUMC), mean residence time (AUMC/AUC), clearance (Cl_B/F=Dose/AUC), volume of distribution ($Vd_{(area)}$/F= Dose/AUC*β) and elimination half life ($t_{1/2}$ = 0.693/β) were calculated by non-compartmental model using MS Excel. The absorption rates were slower than the elimination rate. Hence, based on the principle of flip-flop kinetics, slope of absorption line was used to calculate extrapolated value of $AUC_{0-\infty}$ $AUMC_{0-\infty}$(Bialer *et al*., 1986). The peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were taken as observed. Absolute bioavailability could not be measured since the intravenous administration of tilmicosin was not possible. Hence relative bioavailability was determined as ratio of AUC of drinking water to the AUC of 'in-crop' administration.

Statistics

The effect of different routes ('in-crop' and 'in-water') on the mean plasma tilmicosin concentrations and pharmacokinetic parameters was compared using Mann Whitney U test (SPSS version 20).

RESULTS AND DISCUSSION

In phase I study, the mean plasma concentration of tilmicosin was detectable at 30 minutes and 2 h in 'in-crop' and 'in-water' groups respectively and maintained above reported MIC (0.05 µg/ml) against *M. gallisepticum* (Abd El-Ghany, 2009) up to 24 h (Table 1, Fig 1). Statistically, the plasma tilmicosin concentrations in 'in-water' group was significantly lower ($P<0.05$) than 'in-crop' group at specific time points of 3, 4 and 8 h (Table 1). C_{max} was significantly lower ($P<0.05$) and the t_{max} was significantly prolonged ($P<0.01$) in the 'in-water' group compared to the crop route (Table 2). This might be the consequence of varied consumption pattern of

medicated water resulting in gradual absorption and distribution of the drug, unlike bolus dosed 'in-crop' group.

Although the relative bioavailability of tilmicosin in 'in-water' route was only 52.8 % in comparison with 'in-crop' route, the desirable plasma concentrations achieved indicate a satisfactory regimen. In an earlier study, tylosin was found to be ineffective after 'in-water' administration of tylosin due to very low undetectable concentrations in plasma and very poor bioavailability (Anusha Chinta, 2014). In comparison, tilmicosin is more reliable for therapy through drinking water.

There were no significant differences in the pharmacokinetic parameters such as AUC_{0-t}, $AUC_{0-\infty}$, $AUMC_{0-t}$, $AUMC_{0-\infty}$, MRT and Vd_{area}/F between the two groups. However $t_{1/2}$ and Cl_B/F were significantly different ($P<0.01$) (Table 2). The clearance had increased in the 'in-water' group which could account for the reduced half life. Increased clearance in 'in-water' group could be attributed to the lower bioavailability of 52.8% compared to 'in-crop' route. It is to be noted that absolute clearance could not be calculated.

The pharmacokinetic parameters of 'in-crop' group in the present study were compared to available reports in same species and route of administration. The mean $AUC_{0-\infty}$ is 16.25 ± 3.90 µg.h.ml^{-1} which is less than the reported values such as 24.2 ±3.9 µg.h.ml^{-1} (Abu-Basha *et al*., 2007) and 23.7±4.15 µg.h.ml^{-1} (Elbadawy and Mohamed Aboubakr, 2017). The MRT (11.77 ± 1.04 h) is lesser than other reported values of 71.20 ± 12.87 h (Abu-Basha *et al*., 2007) and 68.4±12.87 h (Elbadawy and Mohamed Aboubakr, 2017). The differences might be due to variation in commercial tilmicosin preparation and bird strains used for the study. The elimination half-life (23.75 ± 5.59 h) in the present study is comparable with 29.3 ± 2.6 h reported by Rassouli *et al* (2016).

Integration of pharmacokinetic and pharmacodynamic parameters for optimization of dosage regimen

The ultimate purpose of a PK study is to compute the dosage regimen with the given parameters obtained. The effectiveness of the dosage regimen is assessed by the integration of PK/PD parameters. For time dependent antibiotics like macrolides, the surrogate parameter 'T>MIC' is the best surrogate indicator of activity for assessing clinical efficacy of tilmicosin. This means that the plasma concentration should be maintained above MIC value for a given duration. A value of T>MIC for more than 50-60% of the treatment duration will effectively control the infection (McKellar *et al*, 2004). In this study, based on the reported MIC of 0.05 µg/ml for *Mycoplasma galiisepticum* (Abd El-Ghany, 2009), T>MIC is maintained for 12 hours (50% of treatment period) indicating satisfactory therapeutic efficacy in both groups.

Residue status in tissues

In the phase II study, detectable concentrations of tilmicosin were found up to 22 days in Lung, Liver and Kidney and up to 18 days in breast and thigh muscle (Table 3).

Higher level of tilmicosin in the lungs is advantageous as this is the site of predilection of Mycoplasma organism. In a previous study, tilmicosin concentration in lung lasted till 14th day after intravenous administration (10 mg/kg b.wt.) and 28th day after subcutaneous administration in sheep (Arooba, 2011). Other studies in different species (swine and sheep) also demonstrated that tilmicosin is therapeutically effective in treating respiratory diseases (Moore *et al*., 1996; Christodoulopoulos *et al*., 2002).

Abd El-Ghany (2009) compared the efficacy of tiamulin (MIC: 0.1 µg/ml) and tilmicosin (MIC: 0.05 µg/ml) against

Table 1: Mean plasma concentrations (Mean ± S.E) of tilmicosin after administration through 'in-crop' (25 mg/kg) and 'in-water' (40 mg/kg) route; (n = 8).

Time (h)	Plasma concentration (µg/ml)	
	In-crop	In-water
0	-	-
0.08	-	-
0.25	-	-
0.5	0.050 ± 0.03	-
1	0.110 ± 0.06	-
2	0.174 ± 0.01	0.050 ± 0.02
3	0.291 ± 0.06	0.108 ± 0.03*
4	0.422 ± 0.05	0.156 ± 0.03*
8	0.423 ± 0.06	0.271 ± 0.03*
12	0.318 ± 0.04	0.317 ± 0.06
24	0.052 ± 0.01	0.087 ± 0.04
48	-	-

*P<0.05, relative to 'in-crop' group.

Table 2: Pharmacokinetic parameters (Mean ± S.E) of tilmicosin after administration through 'in-crop' and 'in-water'; (n=8).

Parameters	Units	In crop	In water
		Dose - 25mg/kg	Dose - 40mg/kg
C_{max}	µg.ml^{-1}	0.51 ± 0.04	0.37 ± 0.03*
t_{max}	h	6.00 ± 1.19	15.00 ± 2.13**
$t_{1/2}$	h	23.75 ± 5.59	9.91 ± 3.03**
AUC_{0-t}	µg.h.ml^{-1}	6.01 ± 0.84	3.48 ± 0.85
$AUC_{0-\infty}$	µg.h.ml^{-1}	16.25 ± 3.90	8.58 ± 2.47
$AUMC_{0-t}$	µg.h^2.ml^{-1}	45.86 ± 7.54	38.32 ± 12.87
$AUMC_{0-\infty}$	µg.h^2.ml^{-1}	189.45 ± 45.64	133.40 ± 56.97
MRT	h	11.77 ± 1.04	12.62 ± 1.66
Cl_B/F	ml.min^{-1}.kg^{-1}	2.21 ± 0.49	7.00 ± 1.50**
$V_{d\ area}/F$	L.kg^{-1}	53.97 ± 5.68	65.95 ± 7.52
Relative bioavailability (F)	%	—	52.8

*P<0.05; **P<0.01, relative to 'in-crop' group.

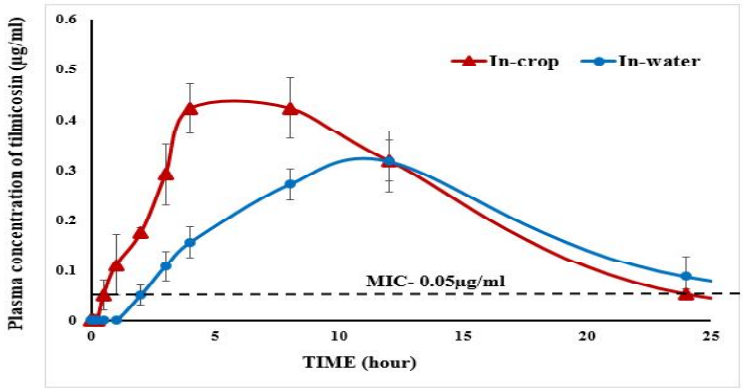
Abbreviation: C_{max}: maximum plasma concentration; T_{max}: time to reach peak plasma concentration; $t_{1/2}$: half- life; AUC_{0-t}: Area under curve from 0 to tth hour; $AUC_{0-\infty}$: Area under curve from 0 to infinity; $AUMC_{0-t}$: Area under moment curve from 0 to tth hour; $AUMC_{0-\infty}$: Area under moment curve from 0 to infinity; MRT, mean residence time; Cl_B/F, Clearance; $V_{d\ area}/F$: volume of distribution.

Table 3: Mean concentrations (mg/kg) (Mean ± S.E) of tilmicosin after administration through drinking water for 5 days at 40 mg/kg b.wt. (n = 3).

Organs	Concentration of tilmicosin (mg/kg) on different days				
	10	14	18	22	25
Lung	3.65 ± 0.12	4.90 ± 0.23	1.01 ± 0.47	1.12 ± 0.40	BDL
Kidney	2.33 ± 2.01	9.49 ± 1.50	6.90 ± 0.61	3.66 ± 1.41	BDL
Liver	11.50 ± 2.43	9.49 ± 1.60	5.91 ± 2.57	0.92 ± 0.31	BDL
Breast muscle	3.32 ± 0.14	1.39 ± 0.83	0.36 ± 0.12	BDL	BDL
Thigh muscle	1.60 ± 1.0	4.09 ± 0.52	1.36 ± 0.60	BDL	BDL

BDL: Below detectable limits.

MRL of tilmicosin in meat, liver and kidney 0.075, 1.0 and 0.25 mg/kg respectively.



(MIC value based on Abd El Ghany, 2009).

Fig 1: Comparison of mean plasma concentrations of tilmicosin after 'in-crop' (25 mg/kg b.wt) and 'in-water' (40 mg/kg b.wt) administration.

Mycoplasma gallisepticum infection in field and revealed that tilmicosin is superior to tiamulin in eradication of infection. Vinothini *et al*. (2019) revealed that the effective concentration of tiamulin in lung was detected only up to 3 days. Thus tilmicosin, owing to its longer persistence in lungs at the dosage used in our study, could be more effective than tiamulin for the control of respiratory disease in broiler chicken.

The Maximum residue levels (MRL) of tilmicosin established by Codex Alimentarius Commission (2015) in chicken meat, liver and kidney are 0.15, 2.4 and 0.6 mg/kg respectively. Accordingly, in our study, tilmicosin concentrations were above reported MRL upto 18th day in meat and liver and upto 22nd day in kidney. As per the manufacturer's recommendations, the withdrawal period is 14 days. The results of our study suggest a longer withdrawal period of at least three weeks. Hence, it will be prudent to reduce the dose of tilmicosin without compromising therapeutic efficacy which might lead to reduced withdrawal period.

CONCLUSION

Administration of tilmicosin through drinking water to broiler chicken at 40 mg/kg b.wt was found to be therapeutically effective with optimum tilmicosin concentration in plasma and lungs. Residues in edible tissues suggest a withdrawal period of three weeks. The results of the study indicate the utility of drinking water as a route of tilmicosin administration against Mycoplasmosis in broiler chicken.

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