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Pharmacokinetics of Tulathromycin in Rabbits after Single Intravenous and Intramuscular Administration

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Abstract: The target of the present study was to investigate the plasma disposition kinetics of tulathromycin in rabbits (n = 20) following a single intravenous (i.v.) bolus or intramuscular (i.m.) injection at a dose rate of 2.5 mg kg^{-1} B.W. using a parallel design. Plasma samples were collected at appropriate times during a 216 h administration interval and were analyzed using a Microbiological Assay Method. The plasma tulathromycin disposition was best fitted to a Two Compartment Open Model after i.v. dosing. The half-lives of distribution and elimination were 0.31±0.07 and 61.83±8.43 h, respectively. The volume of distribution at steady-state was 10.32±1.26 L kg^{-1}, the total body clearance (Cl_{tot}) was 0.13±0.07 L/kg/h and the areas under the concentration-time curves (AUC) was 22.56±2.21 μg/mL/h. Following i.m. administration, the mean $t_{1/2el}$ and AUC values were 103.28±17.65 h and 24.61±2.15 μg/mL/h. The bioavailability was high (102.59±12.82%) with a peak plasma mean concentration (C_{max}) of 0.76±0.11 μg mL^{-1} attained at 0.81±0.06 h (T_{max}). The *in vitro* protein binding percentage was 29.5.

Key words: Tulathromycin, rabbits, pharmacokinetics, protein binding, bioavailability

INTRODUCTION

Rabbits are quite prone to respiratory diseases (Langan *et al*., 2000). The bacteria most often involved in these complications include Pasteurella multocida and *Staphylococcus aureus* (Deeb *et al*., 1990; Broome and Brooks, 1991). However, there are few antibiotics that can provide a safe and effective therapy for such conditions particularly those caused by resistant strains.

Tulathromycin is a new semi-synthetic, injectable triamilide of the macrolide subclass. Macrolide antimicrobials are generally bacteriostatic and act by inhibiting essential protein biosynthesis through selective binding to bacterial ribosomes and stimulating disassociation of peptidyl-tRNA from the ribosome during the translocation process (Vannuffel and Cocito, 1996).

Tulathromycin has favorable antibiotic activity combined with improved large volume of distribution (Nowakowski *et al*., 2004), longer half-life after a single dose than other macrolides (Letavic *et al*., 2002) and concentrated rapidly from the plasma into lung tissue (Benchaoui *et al*., 2004).

Macrolide antibiotics approved for use in livestock include erythromycin, tylosin, spiramycin and tilmicosin. Typically, repeated administration of these products over several days is required to achieve therapeutic or preventative efficacy but single administration therapy is desirable for ensuring a successful response to antimicrobial treatment while minimizing animal handling and maximize compliance, the macrolides, tilmicosin is the only agent used in cattle with an indication of single injection therapy for respiratory disease (Nowakowski *et al*., 2004). Early developmental work showed that the triamilides have potent in vitro activity against strains of *Pasteurella multocida* pathogenic to cattle and swine (Letavic *et al*., 2002).

There have been no previous reports for tulathromycin pharmacokinetics in rabbits. In view of the marked species variations in the kinetic data for antimicrobial drugs, the present study was undertaken to determine the disposition kinetics of tulathromycin in rabbits after a single intravenous and intramuscular administration of 2.5 mg kg^{-1} B.W. in addition, to estimate intramuscular systemic bioavailability of tulathromycin. An appropriate dosage regimen of tulathromycin in rabbits was then derived based on the kinetic parameters.

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MATERIALS AND METHODS

Drugs and chemicals: Draxxin® (20 mL vial solution each mL contains 100 mg of tulathromycin) was obtained from Pfizer, Animal Health Division, Cairo, Egypt and Mueller-Hinton agar (Mast Group Ltd. Merseyside, UK).

Animals: Researchers used twenty New Zealand white rabbits of both sexes, 10-12 months old and weighing 2.350-3.200 kg. The rabbits were housed individually in cages under a 12 h light/dark cycle and fed good quality hay (alfalfa) and/or a pelleted feed concentrate (fiber 18%, protein 14%, calcium>1 and fat 2%) with free access to water. The room temperature and relative humidity were maintained at 20 and 22°C and between 30 and 60%, respectively. The animals were allowed to acclimatize and did not receive any drug treatment for at least 15 days preceding the study.

The study was reviewed and approved by the Institutional Animal Care and Use Committee at Faculty of Veterinary Medicine.

Drug administration: The study was performed as a parallel design with a single treatment period to avoid the physiological changes in young and rapidly growing animals which may alter the pharmacokinetics of the drug between the first and second period as in the case of a cross-over design.

Ten rabbits were given a single intravenous injection into the left ear vein at dose of 2.5 mg kg^{-1} body weight tulathromycin and the other ten were injected intramuscularly into the left semimembranous muscle with the drug at the same dose. Blood samples (0.5 mL each) were taken via indwelling catheter into heparinized vacutainers (Becton Dickinson Vacutainer Systems, Rutherford, NJ, USA) from the right ear vein at 0 (blank sample), 0.083, 0.166, 0.33, 0.5, 0.75, 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 168, 192 and 216 h after treatment. All the blood samples were centrifuged at 3000 g for 10 min to separate the plasma. The plasma samples were frozen at -20°C until analysed.

Analytical method: Quantization of tulathromycin in plasma samples was accomplished by a modified Agar Diffusion Bioassay Method previously reported by Bennett *et al.* (1966) using *Sarcina lutea* (ATCC 9341) as the reference organism. The microbiological assay does not distinguish the parent compound from the possible active metabolites and hence all biologically active substances are detected in microbiological assay and this total activity which could be more useful for pharmacodynamic evaluations than a chemical method (McKellar *et al.*, 1999). A linear relationship existed between the zone of inhibition and the logarithm of tulathromycin concentrations in plasma with a correlation coefficient of 0.997. The limit of quantification for the plasma was 0.02 μg mL^{-1}. The mean percentage recovery of tulathromycin (measured by comparing zone of inhibitions of the spiked samples with external standards in phosphate buffer saline) from plasma was 95%. The intra-assay and inter-assay variations coefficient were <4.3 and 4.5 for plasma, respectively.

Protein binding: The extent of protein binding was determined *in vitro* according to the method described previously by Craig and Suh (1991). This method was based on the diffusion of free antibiotic into the agar medium. The differences in the diameters of the inhibition zones between the solutions of the drugs in the buffer and plasma samples were then calculated.

Pharmacokinetic analysis: A computerized curve-stripping program (R Strip; Micromath Scientific Software, Salt Lake City, UT, USA) was used to analyze the concentration-time curves for each individual animal following the administration of tulathromycin. For intravenous injection, the appropriate pharmacokinetic model was determined by the visual examination of individual concentration-time curves and by application of Akaike's Information Criterion (AIC) (Yamaoka *et al.*, 1978). The distribution and elimination half-lives ($t_{1/2\alpha}$ and $t_{1/2\beta}$, respectively) and the volume of distribution at steady-state (V_{dss}) were calculated using standard equations (Gibaldi and Perrier, 1982).

Tulathromycin plasma disposition curves after intramuscular administration was analyzed following the same procedure as used for intravenous analysis. Each individual curve of tulathromycin over time was analyzed in order to determine the peak concentration (C_{max}) and the time to peak concentration (T_{max}). The program also calculated the non-compartmental parameters using the Statistical Moment Theory (Yamaoka *et al.*, 1978). The terminal elimination half-life ($t_{½el}$) and absorption half life ($t_{½ab}$) were calculated as $ln2/K_{el}$ or $ln2/K_{ab}$, respectively.

The area under plasma concentration-time curve (AUC) and Area Under the first Moment Curve (AUMC) were calculated by the method of trapezoids and extrapolation to infinity was performed. The systemic clearance as Cl = Dose/AUC. The absolute bioavailability (F) was calculated as $AUC_{i.m.}/AUC_{i.v.} \times 100$.

Statistical analysis: The statistical analysis was performed using the SPSS® 17.1 Software package (SAS, Cary, NC, USA). Results are presented as mean±SD. The

non-parametric Wilcoxon test was used to compare the parameters obtained after i.v. and i.m. Means were considered significantly different at $p<0.05$.

RESULTS

Clinical examination of all animals before and after each trial did not reveal any abnormalities. None of the rabbits had treatment related adverse effects during the study. Akaike's Information Criterion test indicated that a two-compartment model best represented the plasma concentration versus time data after i.v. and i.m. administration of tulathromycin in rabbits.

The Mean±SD plasma concentration-time profiles of tulathromycin following single i.v. and i.m. administrations of 2.5 mg kg^{-1} B.W. are illustrated in Fig. 1.

Mean±SD values of pharmacokinetic parameters estimated from the curve fitting are shown in Table 1. Statistical analysis of the plasma pharmacokinetic parameters revealed significant differences in the distribution (absorption) rate constant, distribution (absorption) half-life, elimination half-life, Area Under the Curve (AUC) and MRT between i.v. and i.m. administration.

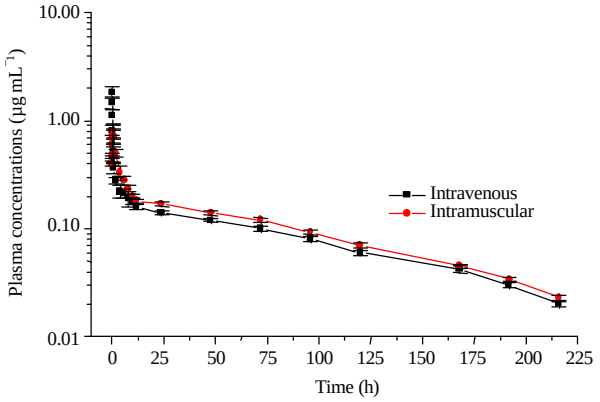

Fig. 1: Mean±SD plasma concentrations of tulathromycin after intravenous ( ) and intramuscular ( ) injection of 2.5 mg kg^{-1} B.W. in rabbits (n = 10)

Table 1: Mean±SD plasma pharmacokinetic parameters of tulathromycin in rabbits (n = 10) following i.v. and i.m. administration at a dose rate of 2.5 mg kg^{-1} B.W.

Parameters	Unit	i.v.	i.m.
α (k_{ab})	h^{-1}	2.51±0.16	0.20±0.03***
$t_{1/2\alpha}$ ($t_{1/2ab}$)	h	0.31±0.07	3.27±0.27***
β(k_{el})	h^{-1}	0.012±0.006	0.01±0.004
$t_{1/2\beta}$ ($t_{1/2el}$)	h	61.83±8.43	103.28±17.65***
V_{dss}	L kg^{-1}	10.32±1.26	-
Cl_{tot}	L/kg/h	0.13±0.070	-
AUC	µg/mL/h	22.56±2.21	24.61±2.15*
MRT	h	85.61±12.36	121.17±19.64***
C_{max}	µg mL^{-1}	-	0.76±0.11000
T_{max}	h	-	0.81±0.06000
F	%	-	102.59±12.82

β(k_{el}): Elimination rate constant; α(k_{ab}): Distribution (absorption) rate constant; $t_{1/2\alpha}$: Distribution half-life; $t_{½ab}$: Absorption half-life; $t_{1/2\beta}$($t_{½el}$): Elimination half-life; V_{dss}: Volume of distribution; Cl_{tot}: Total body clearance; AUC: Area Under the Curve from zero to infinity by the trapezoidal integral; MRT: Mean Residence Time; C_{max}: Maximum plasma concentration; T_{max}: Time to peak concentration; F (%), bioavailability. *$p<0.05$, ***$p<0.001$

Bioavailability of tulathromycin after i.m. administration was 102.59%. *In vitro* plasma protein binding of tulathromycin plasma ranged from 27.3-31.6% with an average of 29.5%.

DISCUSSION

In the current investigation, researchers measured the residual concentrations of tulathromycin in the plasma of rabbits using the microbial inhibition test. The reasons that researchers selected the bioassay are: the bioassay measures the total activity which could be more useful for pharmacodynamic evaluations than HPLC (McKellar *et al*., 1999); congruent results between the data determined by the microbiological assay and those determined by HPLC (Auten *et al*., 1991; Bottcher *et al*., 2001); the Bioassay Method is precise, reproducible and does not require specialized equipment or toxic solvents (Da Silveira Ev and Schapoval, 2002). For these reasons, the application of the microbiological assay for measuring tulathromycin concentration is suitable. The pharmacokinetics profile of tulathromycin in rabbits is reported here for the first time.

The present investigation revealed that plasma tulathromycin concentrations versus time decreased in bi-exponential manner following intravenous and intramuscular injection, demonstrating the presence of distribution and elimination phases and justifying the use of Two Compartmental Open Model for analyzing data. This conclusion is in agreement with that found in previous study of tulathromycin carried out in calves (Tohamy *et al*., 2011).

Following intravenous injection, tulathromycin was rapidly distributed with a short distribution half-life time $t_{½\alpha}$ of 0.31±0.07 h. Similar result was reported for calves 0.17 h by Tohamy *et al*. (2011). The elimination half-life $t_{½\beta}$ of tulathromycin following i.v. administration was 61.83 h, similar result was reported for swine 67.5 h (Benchaoui *et al*., 2004). This value is longer than that reported by Tohamy *et al*. (2011) in calves, 48.35 h. The long plasma half life of tulathromycin in rabbits suggests that tulathromycin may be a rational choice as a single injection.

The lipophilic nature and the limited degree of ionization that characterize the macrolides lead to extensive drug penetration into tissues and fluids and result in large volumes of distribution (Peters *et al*., 1992;

Zhanel *et al*., 2001). These characteristics are also evident in (and are most likely enhanced for) the triamilides due to their amphiphilic nature (Nowakowski *et al*., 2004), the good tissue diffusion may be also related to its low molecular weight or low protein binding (Nix *et al*., 1991). Tulathromycin was widely distributed with a large volume of distribution V_{dss} of 10.32 L kg^{-1} in rabbits. The same magnitude has been reported after i.v. administration of tulathromycin to cattle and swine 11.00 and 13.2 L kg^{-1}, (Nowakowski *et al*., 2004; Benchaoui *et al*., 2004), respectively and higher than that reported by Tohamy *et al*. (2011) in calves 4.52 L kg^{-1}, physiological and age differences may account for this result. A high volume of distribution appears to be typical for the group of macrolides.

Tulathromycin had low body clearance rate CL_{tot} of 0.13±0.07 L/kg/h after intravenous injection in rabbits, the tulathomycin clearance in calves and swine were 0.1 and 0.18 L/kg/h (Tohamy *et al*., 2011; Benchaoui *et al*., 2004), respectively. Such differences could be attributable to interspecies variations in drug metabolism and elimination.

Following intramuscular injection of tulathromycin to rabbits at dose of 2.5 mg kg^{-1}, researchers found that tulathromycin was rapidly absorbed as indicated by large absorption rate constant (k_{ab}) 0.2 h^{-1} and short absorption half-life ($t_{½ab}$) 3.27 h. Tulathromycin was slowly eliminated from the body as evidenced by long elimination half life ($t_{½el}$) and Mean Residence Time (MRT), 103.28 and 121.17 h, respectively.

The elimination half-life $t_{½el}$ of tulathromycin following i.m. administration was 103.28 h, similar result was reported for foals 105 h (Venner *et al*., 2010). This value is longer than that reported by Tohamy *et al*. (2011) in calves, 68.93 h. The long plasma half life of tulathromycin in rabbits support that tulathromycin may be a rational choice as a single injection.

Like all macrolides tulathromycin has low plasma level with calculated C_{max} of 0.76±0.11 μg mL^{-1} at t_{max} of 0.81±0.06 h. For comparison, mean C_{max} values for tulathromycin reported were: in swine 0.62 μg mL^{-1} at 0.25 h (Benchaoui *et al*., 2004), foals 0.464 μg mL^{-1} at t_{max} of 2.47 h (Venner *et al*., 2010), calves 0.33 μg mL^{-1} at 1.12 h (Tohamy *et al*., 2011). Azithromycin which is the parent drug of tulathromycin, reached the mean C_{max} of 0.26 μg mL^{-1} in rabbits (Escudero *et al*., 2006).

The bioavailability of tulathromycin in rabbits after intramuscular injection was high and reached (102.59±12.82%), indicated good absorption of the drug from the site of intramuscular injection. The intramuscular systemic bioavailability has been reported to be 87% in pigs (Benchaoui *et al*., 2004), 82.8% in calves (Tohamy *et al*., 2011). Tulathromycin after intramuscular administration is rapidly and nearly completely absorbed from the injection site to reach maximal plasma concentrations within 1 has reported by Benchaoui *et al*. (2004), Galer *et al*. (2004), Nowakowski *et al*. (2004) and Scheuch *et al*. (2007).

These findings were confirmed by the high AUC of tulathromycin in rabbits following intravenous and intramuscular injection of 2.5 mg kg^{-1} B.W. The AUC of tulathromycin after intravenous injection was 22.56±2.21 μg/mL/h and after intramuscular injection was 24.61±2.15 μg/mL/h, a similar finding reported for tulathromycin in foals 20.5 μg/mL/h (Venner *et al*., 2010), while it is lower than that reported in calves by Tohamy *et al*. (2011) following both routed of administration, 40.95 and 33.91 μg/mL/h, respectively.

Tulathromycin plasma protein binding percent was 29.5%. This value is lower than that reported by Tohamy *et al*. (2011) in calves, 38.86% which could be attributed to species variations.

In general, for macrolides, the time in which the concentration remains above the MIC (T>MIC) is the PK/PD parameter that is most highly correlated with clinical efficacy (Van Bambeke and Tulkens, 2001). However, it is important to note that PK/PD predictive indices determined *in vivo* are based on unbound serum drug concentrations and not on total serum or total tissue concentration which is important for an antimicrobial such as tulathromycin (Toutain and Lees, 2004). These previous PK/PD surrogate markers should be used with caution in the cases of macrolides for which tissue kinetics plays an important role in predicting clinical efficacy.

At present, the Minimum Inhibitory Concentrations (MIC) of tulathromycin against pathogens that affect rabbits have not been reported. Even if available, the $AUC_{24\ h}$/MIC ratio would not assure a good prediction of clinical efficacy for a macrolide such as tulathromycin. Therefore, further studies are needed for a better integration of PK and PD parameters to predict the clinical efficacies of macrolides (Benchaoui *et al*., 2004).

CONCLUSION

The pharmacokinetic results revealed that tulathromycin administered to rabbits by intramuscular route demonstrated rapid absorption with a high bioavailability and long elimination half life ($t_{½el}$). These pharmacokinetic attributes are highly appropriate for an antimicrobial drug indicated for the treatment of bacterial and mycoplasmal respiratory diseases in rabbits. As a result of the previous pharmacokinetic characters it's

obvious that a full course therapy can be obtained after a single dose of tulathromycin and this is advantageous for ensuring a successful treatment outcome when used for prevention or treatment systemic diseases in rabbits where it is desirable to minimize stress induced by repeated handling, particularly when animals are debilitated by respiratory disease.

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