Pharmacokinetics and bioavailability of tulathromycin following intravenous, intramuscular and subcutaneous administrations in healthy rabbits

K. Abo-El-Sooud, N. A. Afifi, A. M. Abd-El-Aty

Pharmacology Department,
Faculty of veterinary Medicine, Cairo University, Giza, Egypt
Corresponding author: Khaled Abo-EL-Sooud, E-mail: kasooud@yahoo.com
Fax: 0020235725240 – 002035710305.
Received:01-03-2012, Accepted: 06-03-2012, Published Online: 17-04-2012
doi: 10.5455/vetworld.2012.424-428

Abstract

Aim: This work was performed to investigate the pharmacokinetics of the triamilide antibiotic, tulathromycin in healthy rabbits.

Materials and Methods: Ten rabbits in each group were given a single dose of 2.5 mg/kg body weight (bw) of tulathromycin via intravenous (IV), intramuscular (IM) and subcutaneous (SC) administrations. The concentration of tulathromycin in plasma was determined by microbiological assay *Bacillus subtilis ATCC* 6633 as the test organism.

Results: Following IV administration, the total body clearance (Cl_{tot}) was 321.70 ml/kg·h, the volume of distribution at steady-state (V_{dss}) was 13.26 L/kg and the value of the elimination half-life ($t_{1/2\beta}$) was 29.29 h. After SC administration, the elimination half-life ($t_{1/2el}$), mean residence time (MRT) and maximum plasma concentration (C_{max}) were significantly higher (36.22 h, 52.54 h and 882.19 ng/ml) than after IM route (31.69 h, 45.89 h and 714.72 ng/ml), respectively. Tulathromycin was bound to the extent of 36% to plasma protein of healthy rabbits. The absolute bioavailabilities were 88.07 and 94.25% after IM and SC injections.

Conclusion: Thus a single dose of tulathromycin is promising treatment for most respiratory disease in rabbits.

Key words: Pharmacokinetics, tulathromycin, bioavailability, rabbits.

To cite this article: Abo-El-Sooud K, Afifi NA, Abd-El-Aty MA (2012) Pharmacokinetics and bioavailability of tulathromycin following intramuscular and subcutaneous administrations in healthy rabbits, *Vet World*, 5(7): 424-428, doi: 10.5455/vetworld.2012.424-428

Introduction

Snuffles is considered the most common infectious respiratory disease observed in pet rabbits [1]. Common clinical signs include nasal discharge, sneezing and conjunctivitis [2,3]. The most commonly agent implicated with these symptoms is *Pasteurella multocida* [4,5]. However, other pathogens are cited, such as *Staphylococcus* spp. and *Bordetella bronchiseptica*, although apparently this is by no means an exhaustive list [1]. Macrolide antibiotics are antibacterial agents that are widely used in the treatment of infections of the respiratory system, soft tissue and skin in humans and domestic animals [6]. Tulathromycin, like other macrolides, binds to the 50S subunit of bacterial ribosomes and thereby inhibits protein synthesis, leading to inhibition of cell division and cell death. Although macrolides are generally regarded as bacteriostatic, tulathromycin actually exhibits mixed bacteriostatic and bactericidal activity [7].

Repeated administration of these products over several days is typically required to achieve a therapeutic effect. Prolonged exposure to antibiotics is important for the treatment and/ or prevention of several diseases in rabbits, however single administration therapy is desirable for producers that wish to minimize animal handling. Tulathromycin is a novel triamilide antibiotic approved for the treatment of respiratory diseases in animals. It may be retained in the lung for many days after administration of a single dose [8-10]. The drug has a long plasma elimination half-life in cattle of 90 h [7,11], and therapeutic concentrations have been detected in lung tissue for 10 to 15 days after a single dose [11,12]. Therefore, tulathromycin possibly will be recommended for use in rabbits for several respiratory diseases including pneumonia. To our knowledge there have not been any reports published on the pharmacokinetics of tulathromycin in rabbits. Consequently, the aim of the present study was to investigate the pharmacokinetic profile of tulathro-

mycin in rabbits following a single IV, IM and SC injections to estimate an appropriate dosage regimen of tulathromycin in rabbits.

Materials and Methods

Drug: Tulathromycin was used as 10% injectable solution (Draxxin®, Pfizer Animal Health, New York, NY), Each ml of Draxxin® contains 100 mg of tulathromycin as the free base in a 50% propylene glycol vehicle, monothioglycerol (5 mg/ml), with citric and hydrochloric acids added to adjust pH.

Animals and Husbandry: Thirty healthy New Zealand white rabbits of both sexes, 10-12 months old and weighing 2.7-3.5 kg were used. Rabbits were housed separately in individual 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^{0}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 experiments were carried out according to the National regulations on animal welfare and institutional animal Ethical Committee (IAEC).

Experimental design: The animals were allocated to three groups of 10 rabbits each. Rabbits were individually weighed before drug administration and doses were calculated precisely. Rabbits of all groups of groups were injected a at a dose of 2.5 mg/kg bw of 10% solution of tulathromycin intravenously into the left ear vein, intramuscularly into the left semi-membranous muscles and subcutaneously in the scruff using graduated 1 ml syringes, respectively.

Blood samples from the all groups (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), 0.25, 0.33, 0.5, 0.75, 1, 2, 4, 6, 8, 10, 12, 18, 24, 48 h and everyday for 10 days after all routes of injections. Plasma was separated by centrifugation at 2000***g*** for 10 min and stored at -20 ^{0}C until assayed.

Assay for Tulathromycin: Tulathromycin concentrations in plasma were determined by a microbiological agar plate assay [13] using *Bacillus subtilis ATCC* 6633 as the test organism. Standard curves of tulathromycin (Pfizer Animal Health, New York, NY) were prepared in pooled antibacterial-free plasma. All samples were directly added to the culture plate. The limit of quantitation by this method was 20 ng/ml in plasma. The response of tulathromycin was linear over the range of concentration between 20 and 2000 ng/ml with a correlation coefficient (r^2) of 0.998. The intra-assay coefficient of variation rate of elimination (CV) was 8%. Negative control samples (non-treated) showed no bacterial inhibition, indicating no intrinsic antibacterial activity of the samples.

In vitro plasma protein binding: The extent of protein-binding was determined *in vitro* using the method of Craig & Suh [14] which is based on the diffusion of the free antibiotic into the agar medium. The drug was dissolved in phosphate buffer (pH 6.2) and antibiotic-free rabbit's plasma at concentrations of 200, 400, 600, 800 and 1000 ng/ml. The differences in the diameter of the inhibition zone between the solutions of the drugs in the buffer and plasma were calculated. The percentage of protein bound fraction was calculated according to the following equation:

Protein binding % = Zone of inhibition in buffer – Zone of inhibition in Plasma × 100 / Zone of inhibition in buffer

Pharmacokinetic analysis: Plasma concentrations of tulathromycin after IV, IM and SC administrations were subjected to a compartmental analysis using a nonlinear least-squares regression analysis with the help of a computerized curve-stripping software package (R Strip; Version 5.0; Micromath Scientific Software, Salt Lake City, UT, USA). Data were examined by sequential weighted nonlinear regression. Monoexponential, biexponential and triexponential equations were fitted to individual plasma concentration–time data. Akaike's Information Criterion (AIC) [15], residual sum of squares (Rs) and analysis of residual's plots were used to discriminate between models [16]. The distribution and elimination half-lives ($t_{1/2}$ and $t_{1/2}$), the volume of distribution at steady state (V_{dss}) were calculated according to standard equations [17]. The total body clearance was calculated as Cl_{tot} = Dose/ AUC. Statistical moments were used to compute the non-compartmental model of area under the concentration–time curves (AUC), area under the first moment curve (AUMC); mean residence time (MRT) and bioavailability (F), where:

$F = [\text{mean AUC}_{\text{IM or SC}} / \text{mean AUC}_{\text{IV}}] \text{ X } 100.$

Statistical analysis: The statistical analysis was performed using the SPSS® 10.0 software package (SAS, Cary, NC, USA). Results are presented as arithmetic mean ± standard errors (SE). The nonparametric Wilcoxon test was used to compare the

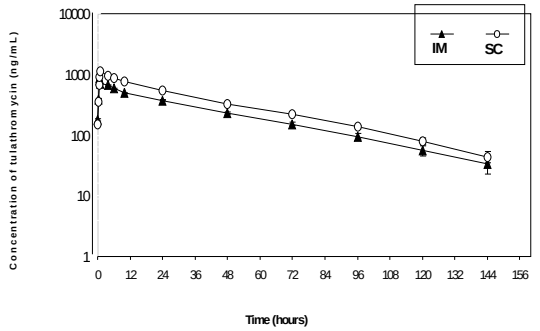

Figure-1. Mean ± SE of plasma concentrations of tulathromycin in rabbits after SC and IM administrations of 2.5 mg/kg b.w. (n=10)

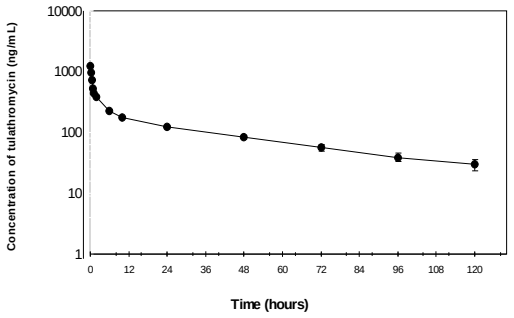

Figure-2. Mean ± SE of plasma concentrations of tulathromycin in rabbits after IV injection of 2.5 mg/kg b.w. (n=10)

Table-1: Mean ± SE plasma pharmacokinetic parameters of tulathromycin in normal rabbits (n=10) following IV, IM and SC administrations at a dose rate of 2.5 mg/kg bw.

Parameters	Unit	IV	IM	SC
$t_{1/2\ alpha}$	h	0.50 ± 0.10	---	---
$t_{1/2ab}$	h	---	0.11 ± 0.01	0.20 ± 0.02**
$t_{1/2beta}$	h	29.29 ± 5.30	---	---
$t_{1/2el}$	h	---	31.69 ± 1.80	36.22 ± 2.26
V_{dss}	L/kg	13.26 ± 2.10	---	---
Cl_{tot}	ml/kg/h	321.70 ± 15	---	---
$AUC_{0-\infty}$	ng/h/ml	7771.7 ± 287	6844.7± 252	7324.5 ± 304
AUMC	$ng/h^2/ml$	262855 ± 723	231501± 655	247729 ± 578
MRT	h	37.25 ± 2.45	45.89 ± 2.25	52.54 ± 2.85*
C_{max}	ng/ml	---	714.72 ± 24	882.19 ± 30**
T_{max}	h	---	1.00 ± 0.26	1.55 ± 0.26
F	%	---	88.07 + 3.20	94.25 + 4.10

$t_{1/2alpha}$: distribution half-life; $t_{½ab}$: absorption half-life; $t_{1/2}$ ($t_{½el}$): elimination half-life; V_{dss}: volume of distribution; Cl_{tot}: total body clearance; AUC: area under the curve by the trapezoidal integral; AUMC: area under moment curve by the trapezoidal integral; MRT: mean residence time; C_{max}: maximum plasma concentration; T_{max}: time to peak concentration; F%: bioavailability. Values of diseased rabbits were significantly different from corresponding normal rabbits at $^{*}P<0.05$, $^{**}P<0.01$.

parameters obtained in healthy and diseased rabbits following each route of administration. Means were considered significantly different at $p< 0.05$ and $P<0.01$.

Results

All animals remained in good health throughout the acclimatization and study periods. No adverse effects were observed in the present studies when the tulathromycin formulation was administered to rabbits at 2.5 mg/kg bw following IV, IM and SC administrations**.** Mean (±SE) tulathromycin plasma concentration–time curves in healthy rabbits following IV, IM and SC injections are plotted on a semi-logarithmic graph in Figures 1&2. Values of pharmacokinetic parameters are presented in Table 1. Following a single IV route the plasma concentration time curve of tulathromycin was best fitted to follow a two compartment open model. The total body clearance (Cl_{tot}) was 321.70 ml/kg·h, the volume of distribution at steady-state (V_{dss}) was 13.26 L/kg and the value of the elimination half-life ($t_{1/2\beta}$) was 29.29 h. After SC administration, the elimination half-life ($t_{1/2el}$), mean residence time (MRT) and maximum plasma concentration (C_{max}) were significantly higher (36.22 h, 52.54 h and 882.19 ng/ml) than after IM route (31.69 h, 45.89 h and 714.72 ng/ml), respectively.

Tulathromycin was bound to the extent of 36% to plasma protein of healthy rabbits. The absolute bioavailabilities were 88.07 and 94.25% after IM and SC injections, respectively. The slow elimination and extensive distribution following a single dose of tulathromycin are fortunate pharmacokinetic characteristics and promising for the treatment of respiratory disease in rabbits.

Discussion

This study used the microbiological assay for determination of tulathromycin in plasma which does not separate the parent compound from the active metabolites. Tulathromycin is metabolized slowly, and the majority of drug is excreted unchanged in feces and urine [7]. Nevertheless, it measures the total activity which could be more useful for pharmacodynamic evaluations than high performance liquid chromatography (HPLC) methods [18]. In the present study, pharmacokinetics of tulathromycin is characterized in normal healthy rabbits after three routes of administrations. Following IV administration of tulathromycin (2.5 mg/kg bw), the average volume of distribution at steady-state (V_{dss}) is 13.26 L/kg which is typically similar to that reported in swine and larger than that reported in cattle, 11 L/kg [12]. The calculated half-life of elimination following IV administration in rabbits was (29.29 h) shorter than the values reported in swine (67.5 h) by Benchaoui *et al*., [19]. Consequently the systemic clearance of tulathromycin in our study (321.70 L/h/kg) was faster than reported in swine (181 L/h/kg) [19]. Differences in the kinetic parameters are relatively common and frequently related to interspecies variation, age, breed, health status of the animals and/or the assay method used [20]. A basic nature, limited degree of ionization, and lipophilicity are characteristics of macrolides in general, enabling extensive drug penetration into tissues and fluids, and resulting in large volumes of distribution [8,21,22]. Triamilides are basic compounds and include in their structure three amine sites with pK_{a1} = 8.49, pK_{a2} = 9.28 and pK_{a3} = 9.80. These properties allow tulathromycin to achieve high tissue penetration. In this respect, Benchaoui *et al*., [19] stated that tulathromycin is extensively distributed to lung tissue following IM administration and the peak concentrations in the lung were markedly higher (lung C_{max} = 3470 ng/g) than in plasma (C_{max} = 616 ng/mL), and were achieved by 24 h (T_{max}) after injection. Over the time course following administration, drug concentrations in the lung were 24.9 to 181 times higher than those measured in plasma. Also, Evans, [7] stated that tulathromycin is slowly release from cells, are thought to account for the prolonged drug levels in lung and other tissue. Actual lung half-life values for cattle and swine were calculated as 184 and 142 hours, respectively [11,19]. Consequently, it has been suggested that tulathromycin may be safe and effective in treating *P. multocida* and other pulmonary pathogens in rabbits.

After SC administration, the elimination half-life ($t_{1/2el}$), mean residence time (MRT) and maximum plasma concentration (C_{max}) were significantly higher (36.22 h, 52.54 h and 882.19 ng/ml) than after IM route (31.69 h, 45.89 h and 714.72 ng/ml), respectively. These values are lower than reported in domestic goats after SC injection by Clothier *et al*., 2011. The mean time of maximum plasma concentration (T_{max}) following SC injection (1.55 h) is longer than estimated in swine [19] and in cattle [11&23]. Tulathromycin was bound to the extent of 36% to plasma protein of healthy rabbits. The value was similar to the value reported in cattle (40%) by Nowakowski *et al*., [11] and lower than reported in goats (50%) by Clothier *et al*., [24].

The absolute bioavailabilities were 88.07 and 94.25% after IM and SC injections, respectively indicating excellent absorption of tulathromycin from parenteral routes. Similar high values have been obtained for tulathromycin after IM route in swine (87.7%) by Benchaoui *et al*., [19] and after SC route in cattle (91%) by Nowakowski *et al*., [11].

Conclusion

We conclude that a single dose of tulathromycin at 2.5 mg/kg bw is promising for treatment of respiratory disease in rabbits owing to its fortunate pharmacokinetic characteristics which are slow elimination and extensive distribution.

Acknowledgements

The authors acknowledge Pharmacology Department, Faculty of veterinary Medicine, Cairo University for the great help in rearing and handling of rabbits. This work has been performed by the authors of their own interest.

Competing interest

Authors declare that they have no competing interest.

References

1. Langan, G.P. Lohmiller, J.J. Swing, S.P. and Wardrip, C.L. (2000) Respiratory diseases of rodents and rabbits. *The Veterinary clinics of North America. Small animal practice*, 30:1309–1335.
2. Percy, D.H., and Barthold, S.W. (2001) Pathology of laboratory rodents and rabbits. lowa state press. Ames, pp. 315.
3. Boucher, S. and Nouaille, L. (2002) Manuel pratique des maladies des lapins. *France Agricole* 272.
4. Deeb, B.J. DiGiacomo, R.F. Bernard, B.L. and Silbernagel, S.M. (1990) *Pasteurella multocida* and *Bordetella bronchiseptica* infections in rabbits. *Journal of Clinical Microbiology*, 28: 70–75.

5. Broome, R.L. and Brooks, D.L. (1991) Efficacy of enrofloxacin in the treatment of respiratory pasteurellosis in rabbits. *Laboratory animal science*, 41: 572–576.
6. Er, A. Ulutas, E. Altan, F. Cetin, G. Bulbul, A. Elmas, M. and Yazar E. (2011) Tulathromycin disturbs blood oxidative and coagulation status. *African Journal of Biotechnology*, 10: 3243-3247.
7. Evans, N.A. (2005). Tulathromycin: an overview of a new triamilide antimicrobial for livestock respiratory disease. *Veterinary Therapeutics*, 6: 83– 95.
8. Anadon, A. and Reeve-Johnson, L. (1999) Macrolide antibiotics, drug interactions and microsomal enzymes: implications for Veterinary Medicine, *Reseaech in Veterinary Science*, 66: 197-203.
9. Abu-Gharbieh, E. Vasina, V. Poluzzi, E. and De Ponti, F. (2004) Antibacterial macrolides: a drugs class with a complex pharmacological profile. *Pharmacological Research*, 50: 211-222.
10. Yazar, E. (2009) Chemotherapeutics, in: *Veterinary Drug*, Ed: Yazar E, Nobel Press, Istanbul, Turkey, pp. 19-107.
11. Nowakowski, M.A. Inskeep, P.B. Risk, J.E. Skogerboe, T.L. Benchaoui, H.A. Meinert, T.R. Sherington, J. and Sunderland, S.J. (2004) Pharmacokinetics and lung tissue concentrations of tulathromycin, a new triamilide antibiotic, in cattle. *Veterinary Therapeutics*, 5: 60–74.
12. Galer, D. Hessong, S. Beato, B. Risk, J. Inskeep, P. Weerasinghe, C. Schneider, R.P. Langer, C. LaPerle,J. Renouf, D. Bessire, A. Espanol, E. Rafka, R. Ragan, C. Boettner, W. Murphy, T. Keller, D. Benchaoul, H. and Nowakowski, M.A., (2004) An analytical method for the analysis of tulathromycin, an equilibrating triamilide in bovine and porcine plasma and lung. *Journal Agri. Food Chem.*, 52: 2179–2191.
13. Tsai, C and Kondo, F. (2001) Improved agar diffusion method for detecting residual antimicrobial agents. *Journal of Food Protection*, 64: 361–366.
14. Craig, A.W. and Suh, B. (1991) Protein binding and the antibacterial effects. Method for the determination of protein binding. In Antibiotics in laboratory Medicine, 3rd edn (Lorian, V., ED), pp. 367-402. Williams & Wilkins, Baltimore, Maryland, USA.
15. Yamaoka, K. Nakagawa, T. and Uno, T. (1978) Statistical moment in pharmacokinetics. *Journal of Pharmacokinetic and Biopharmaceutics*, 6: 547-558.
16. Powers, J. (1990) Statistical analysis of pharmacokinetic data. *Journal of Veterinary Pharmacology and Therapeutics*, 13: 113–120.
17. Gibaldi, M. and Perrier, D. (1982) Pharmacokinetics, 2nd edn. Marcel Dekker, New York. 409-417.
18. McKellar, Q. Gibson, I. Monteiro, A. and Bregante, M. (1999) Pharmacokinetics of enrofloxacin and danofloxacin in plasma, inflammatory exudate, and bronchial secretions of calves following subcutaneous administration. *Antimicrobial Agents and Chemotherapy*, 43: 1988-1992.
19. Benchaoui, H. A. Nowakowski, M. Sherington, J. Rowan, T.G. and Sunderland, S. J. (2010) Pharmacokinetics and lung tissue concentrations of tulathromycin in swine. *Journal of Veterinary Pharmacology and Therapeutics*, 27:203–210.
20. Haddad, N.S. Pedersoli, W.M. Ravis, W.R. Fazeli, M.H. and Carson, R.L. (1985) Combined pharmacokinetics of gentamicin in pony mares after a single IV and IM administration. *American Journal of Veterinary Research*, 46: 2004-2007.
21. Peters, D.H., Friedel, H.A. and McTavish, D. (1992) Azithromycin: a review of its antimicrobial activity, pharmacokinetics properties and clinical efficacy. *Drugs*,44: 750–799.
22. Zhanel, G.G., Dueck, M., Hoban, D.J., Vercaigne, L.M., Embil, J.M., Gin, A.S. and Karlowski, J.A. (2001) Review of macrolides and ketolides –focus on respiratory tract infections. *Drugs*, 61: 443–498.
23. Kilgore, W.R. Spensley, M.S. Sun, F. Nutsch, R. G. Rooney, K.A. and Skogerboe, T.L. (2005) Therapeutic efficacy of tulathromycin, a novel triamilideanti-microbial, against bovine respiratory disease in feeder calves. *Veterinary Therapeutics*, 6: 143–153.
24. Clothier, K. A. Leavens, T. Griffith, R. W. Wetzlich, S. E. Baynes, R. E. Riviere, J. E. and Tell, L. A. (2011) Pharmacokineticsof tulathromycin after single and multiple subcutaneous injections in domestic goats (*Capra aegagrus hircus*). *Journal of Veterinary Pharmacology and Therapeutics*, 34: 448–454.

* * * * * * * *