Some pharmacokinetic aspects of tulathromycin in Fresian cattle calves

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ABSTRACT: Pharmacokinetics of tulathromycin was studied after single dose intravenous, intramuscular and subcutaneous administration. Six apparently healthy Fresian cattle calves were used in a crossover design with 15-day washout period. After intravenous injection of tulathromycin, the half-lives of distribution and elimination ($t_{0.5(\alpha)}$ and $t_{0.5(\beta)}$), volume of distribution at steady state (Vd_{ss}), mean residence time (MRT) and total body clearance (Cl_B) were 0.166 h., 48.348 h., 4.252 L kg^{-1}., 69.645 h. and 0.061 L kg^{-1} h^{-1}., respectively. Following intramuscular and subcutaneous administration of tulathromycin, the maximum concentration (C_{max}) 0.330 and 0.309 ug ml^{-1} were achieved at a maximum times (t_{max}) 1.118 and 1.234 h., respectively. The mean values for absorption and elimination half-lives ($t_{0.5(ab)}$ and $t_{0.5(el)}$) and MRT were 0.135 and 0.155 h., 68.929 and 65.874 h., 99.562 and 95.165 h., respectively. The intramuscular and subcutaneous bioavailabilities were 82.8 and 71.85%, respectively. The result of *in-vitro* protein-binding study indicated that 38.86 % of tulathromycin was bound to calve's serum proteins. [M.A. TOHAMY, A.A.M. EL-GENDY and Taha A. Attia. Journal of American Science 2011;7(5):651-655]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: pharmacokinetic; tulathromycin; Fresian; cattle; calves

1. INTRODUCTION

Macrolide antibiotics, which are active agents against *Gram-positive* bacteria, are frequently used as veterinary drugs in food-producing animals. They target the bacterial ribosome and inhibit bacterial protein biosynthesis **(Leal et al., 2001)**. Macrolide antibiotics are antibacterial agents used as veterinary drugs in food-producing animals with either a curative or prophylactic aim **(Codony et al., 2002)**.

Tulathromycin is a recently introduced, long acting semi-synthetic macrolide, prescribed for respiratory infections **(Pikkemaat et al., 2009)**, with a large volume of distribution **(Nowakowski et al., 2004)**, a long elimination half-life **(Benchaoui et al., 2004)** and a high concentration in lung tissue after intramuscular injection in cattle and swine **(Galer et al., 2004)**. Tulathromycin is presently approved in European countries and the USA for the treatment of bacterial respiratory diseases in cattle and swine **(Scheuch et al., 2007; Venner et al., 2007)**.

The aim of this study is to investigate the serum concentrations and some pharmacokinetic parameters of tulathromycin after single intravenous (IV), intramuscular (IM) and subcutaneous (SC) administration in Fresian cattle calves. In addition, to estimate intramuscular and subcutaneous systemic bioavailabilities of tulathromycin.

2. MATERIALS AND METHODS

2.1. MATERIALS

2.1.1. Drug

Tulathromycin 100 mg ml^{-1} was supplied as an injectable solution (Draxxin®) by animal health division Pfizer Company, Cairo, Egypt.

2.1.2. Animals

Six apparently healthy, male and female Fresian cattle calves (3-9 months old and mean body weight of 98-123 kg) were used. Animals were obtained from the Animal Farm, Faculty of Veterinary Medicine, Beni-Suef University, kept in hygienic stall under good hygienic condition and fed milk, barseem and tibin with free access to water.

2.2. METHODS:

2.2.1. Experimental protocol

All calves were administered 2.5 mg kg^{-1} tulathromycin **(Schunicht et al., 2007, Venner et al., 2007; Angen et al., 2008; Young et al., 2010)** by intravenous, intramuscular and subcutaneous route with a 2 weeks washout period between each route of administration. Blood samples were collected via vein puncture from jugular vein before and 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96 and 120 hours post-administration. Blood samples were left to clot then centrifuged at 3000 revolution per minute for 15 minutes to obtain clear serum that was kept frozen at -20 °C until assayed.

2.2.2. Drug bioassay

Samples were assayed by microbiological assay according to the method of **Arret et al. (1971)** using *Micrococcus luteus* (ATCC 9341) as a test organism. The determination of antibiotic concentration is commonly carried out by microbiological assays, but they are often lengthy and lack the specificity and precision required for regulatory purposes **(Leal et al., 2001)**. Standard tulathromycin concentrations of 0.156, 0.3125, 0.625, 1.25, 2.5, 5 and 10 ug ml^{-1} were prepared in antibiotic-free calf serum and phosphate buffer saline (pH 8). The minimal detectable limit for the assay method was 0.156 ug ml^{-1}. Semi-logarithmic plots of the inhibition zone diameter versus standard tulathromycin concentrations in serum and phosphate buffer were linear with typical correlation coefficient of 0.998 (for the standard curve). The difference of inhibition zone diameter between the solutions of the drug in serum and buffer was used to calculate the *in-vitro* protein binding tendency of tulathromycin according to **Craig and Suh (1991)** by the following equation:

Protein binding % = Zone of inhibition in buffer-Zone of inhibition in serum x 100
Zone of inhibition in buffer

2.2.3.Pharmacokinetic analysis

Serum concentrations of tulathromycin for each individual calf 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 program (R Strip; Micromath Scientific Software, Salt Lake City, UT, USA). For IV, IM and SC data, the appropriate pharmacokinetic model was determined by visual examination of individual concentration-time curves and by application of Akaike's Information Criterion (AIC) **(Yamaoka et al., 1978)**. Following IV injection, the serum concentration-time relationship was best estimated as a two-compartment open model system **(Baggot, 1978)** according to the following bi-exponential equation: $C_p = Ae^{-\alpha t} + Be^{-\beta t}$, where C_p is the concentration of drug in the serum at time t; A is the intercept of the distribution phase with the concentration axis expressed as ug ml^{-1}; B is the intercept of the elimination phase with the concentration axis expressed as ug ml^{-1}; α is the distribution rate constant expressed in units of reciprocal time (h^{-1}); β is the elimination rate constant expressed in units of reciprocal time (h^{-1}); and e is the natural logarithm base.

After IM and SC administration, data was analyzed by adopting a one-compartment open model. This program also calculated non-compartmental parameters using the statistical moment theory **(Gibaldi and Perrier, 1982)**. The C_{max} (maximum serum concentration) and t_{max} (time of maximum serum concentration) were taken directly from the curve. The terminal elimination half-life ($t_{0.5(el)}$) and absorption half-life ($t_{0.5(ab)}$) were calculated as $\ln 2/K_{el}$ or $\ln 2/K_{ab}$, respectively, where K_{el} and K_{ab} are the elimination and absorption rate constants, respectively. The area under serum 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 mean residence time (MRT) and mean absorption time (MAT) were calculated as MRT = AUMC/AUC and MAT = $MRT_{i.m} - MRT_{i.v}$. The total body clearance (Cl_B) was calculated as Cl_B = Dose/AUC and the absolute bioavailability (F) as F = $AUC_{i.m}/AUC_{i.v} \cdot 100$. Results were expressed as mean and standard error (S.E). Standard errors were calculated from the mean data according to **Snedecor and Cochran (1976)**.

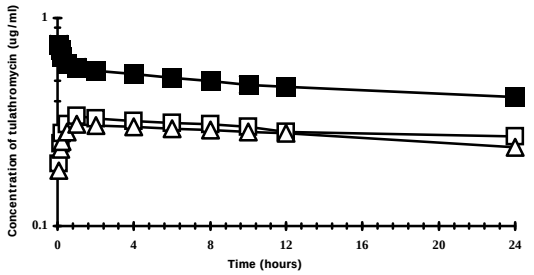

Figure (1): Semi-logarithmic graph depicting the time-concentration of tulathromycin in serum of cattle calves after a single intravenous (■), intramuscular (□) and subcutaneous (Δ) injection of 2.5 mg kg^{-1} b.wt.

3. RESULTS

Disposition of tulathromycin in serum after intravenous injection was best fitted by the 2-compartment open pharmacokinetic model (Figure 1). The pharmacokinetic parameters of tulathromycin following a single intravenous, intramuscular and subcutaneous administration of 2.5 mg kg^{-1} b.wt are recorded in table (1). The results of the present study revealed that tulathromycin was rapidly distributed following intravenous injection in calves as indicated by short ($t_{0.5(\alpha)}$) 0.166 h. The elimination half-life ($t_{0.5(\beta)}$) and total body clearance (Cl_B) were 48.348 h and 0.061 L $kg^{-1}h^{-1}$., respectively. Following intramuscular and subcutaneous administration, the drug was rapidly absorbed with $t_{0.5(ab)}$ of 0.135 and

0.155 h and maximum serum concentrations (C_{max}) of 0.330 and 0.309 ug ml^{-1} were achieved at (t_{max}) of 1.118 and 1.234 h., respectively. The elimination half-lives ($t_{0.5(el)}$) and systemic bioavailabilities were 68.929 and 65.874 h., 82.80 and 71.85 % for tulathromycin given by intramuscular and subcutaneous routes, respectively. The *in-vitro* serum protein-binding tendency was calculated to be 38.86 %.

Table (1): Mean (± SE) kinetic parameters of tulathromycin following a single intravenous (IV), intramuscular (IM) and subcutaneous (SC) administration of 2.5 mg kg^{-1} b.wt in calves (n=6).

Parameter	Unit	IV	Parameter	Unit	IM	SC
C_p^o	ug ml^{-1}	0.825±0.041	C_{max}	ug ml^{-1}	0.330±0.02	0.309±0.06
A	ug ml^{-1}	0.255±0.07	t_{max}	h	1.118±0.10	1.234±0.17
B	ug ml^{-1}	0.570±0.03	K_{ab}	h^{-1}	5.145±0.34	4.464±0.41
α	h^{-1}	4.166±0.22	K_{el}	h^{-1}	0.0101±0.006	0.0105±0.004
β	h^{-1}	0.014±0.002	$t_{0.5(ab)}$	h	0.135±0.07	0.155±0.04
K_{12}	h^{-1}	1.275±0.06	$t_{0.5(el)}$	h	68.929±4.62	65.874±5.26
K_{21}	h^{-1}	2.884±0.058	AUC	ug ml^{-1} h^{-1}	33.905±2.33	29.423±4.23
K_{el}	h^{-1}	0.021±0.008	AUMC	ug ml^{-1} h^{-2}	3309.2±227.6	2832.8±277.1
$t_{0.5(\alpha)}$	h	0.166±0.059	MRT	h	99.562±6.88	95.165±7.34
$t_{0.5(\beta)}$	h	48.348±2.28	MAT	h	29.917±1.87	25.52±2.64
V_c	L kg^{-1}	3.030±0.14	F	%	82.80±5.67	71.85±6.72
Vd_{ss}	L kg^{-1}	4.252±0.284				
Cl_B	L kg^{-1} h^{-1}	0.061±0.007				
MRT	h	69.645±5.07				
AUC	ug ml^{-1} h^{-1}	40.949±3.06				
AUMC	ug ml^{-1} h^{-2}	2774.0± 215.12				

C_p^o concentration at zero time (immediately after single IV injection); A, B zero-time intercepts of the biphasic disposition curve; α, β hybrid rate constants representing the slopes of distribution and elimination phases, respectively; k_{12} first-order constant for transfer from central to peripheral compartment; k_{21} first-order constant for transfer from peripheral to central compartment; K_{el} elimination rate constant; $t_{0.5(\alpha)}$ distribution half-life; $t_{0.5(\beta)}$ elimination half-life; MRT mean residence time; AUC_{0-24} area under serum concentration-time curve; AUMC area under moment curve; Vc apparent volume of the central compartment; Vd_{ss} volume of distribution at steady state; Cl_B total body clearance. k_{ab} first-order absorption rate constant; C_{max} maximum serum concentration; t_{max} time to peak serum concentration; $t_{0.5(ab)}$ absorption half-life; $t_{0.5(el)}$ elimination half-life; MAT mean absorption time; F fraction of drug absorbed systemically after IM injection

4.DISCUSSION

Disposition of tulathromycin in calf's serum after intravenous administration was best described by the two-compartment open-pharmacokinetic model. Tulathromycin was rapidly distributed with a short distribution half-life ($t_{0.5(\alpha)}$) of 0.166 h. Similarly, rapid distribution had been recorded for the tylosin in sheep and goats (0.143 and 0.213 h) **(Taha et al., 1999)**. The apparent volume of distribution at steady-state (Vd_{ss}) is an accurate indication of the diffusion of the drug into the body tissues **(Galinsky and Svensson 1995)**. The result of this study revealed that tulathromycin was widely distributed to extra-vascular tissues as indicated by larger volumes of distribution at steady-state (Vd_{ss}) 4.252 L kg^{-1}. Tulathromycin is widely distributed and its elimination is extremely slow with half-life 4-6 days (**Benchaoui et al., 2004; Galer et al., 2004; Nowakowski et al., 2004**). The drug was widely distributed with volumes of distribution at equilibrium ranging between 12.7 and 18.2 L kg^{-1} and slowly eliminated with half-life 101-158 h **(Scheuch et al., 2007)**.

Serum concentration-time curves describing the disposition of tulathromycin after intramuscular and subcutaneous administration were remarkably similar, as recorded for C_{max}, t_{max}, $t_{0.5(ab)}$ and $t_{0.5(el)}$. In this study, tulathromycin achieved a maximal concentrations (C_{max} 0.330 and 0.309 ug ml^{-1}), relatively close to that reported in foals (0.410 ug ml^{-1}) **(Scheuch et al., 2007)** but lower than that reported in pigs (0.616 ug ml^{-1}) **(Benchaoui et al., 2004)**. Differences in kinetic parameters are relatively common and are frequently related to interspecies variation, age, breed, health status of the animals and/or the assay method used **(Haddad et al., 1985)**.

Absorption was rapid after intramuscular and subcutaneous administration of the drug as indicated by large absorption rate constant (k_{ab}) 5.145 and 4.464 h^{-1} and short absorption half-life ($t_{0.5(ab)}$) 0.135 and 0.155 h., respectively. Following a single subcutaneous injection, the drug was rapidly absorbed **(Nowakowski et al., 2004)**. Tulathromycin was slowly eliminated from the body as evidenced by long elimination half life ($t_{0.5(el)}$) and mean residence time (MRT) 68.929 and 65.874 h., 99.562 and 95.165 h., respectively.

The values of systemic bioavailability of tulathromycin after intramuscular injection 82.80 %, 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)**. Tulathromycin after intramuscular administration is rapidly and nearly completely absorbed from the injection site to reach maximal serum concentrations within 1 h (**Benchaoui et al., 2004; Galer et al., 2004; Nowakowski et al., 2004; Scheuch, 2007**). The *in vitro* protein binding tendency of tulathromycin to calve's serum proteins was 38.86 %. For another macrolide antibiotics (clarithromycin and azithromycin) this value ranged from 7-50 % **(Marzo and Dal Bo 1998)**.

The minimum inhibitory concentrations (MIC_s) for tulathromycin against isolated bovine and porcine respiratory pathogens (*Mannheimia haemolytica, Pasteurella multocida, Mycoplasma bovis* and *Mycoplasma hypopneumoniae*) was previously reported to be 0125-0.25 ug ml^{-1} **(Godinho, 2008)**. The serum concentration of tulathromycin following intravenous, intramuscular and subcutaneous administration was higher than the MIC for the previously mentioned bacteria. This result indicates that tulathromycin could be used successfully for treatment of such types of bacterial infection in calves.

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3/21/2011