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Comparative Pharmacokinetics of Two Injection Formulations of Tulathromycin after a Single Intramuscular Administration in Healthy Swine

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Abstract: The objective of this study was to campare different pharmacokinetic parameters of a locally manufactured (Tulathromycin Injection, CONTINENT, China) and reference (Draxxin, Pfizer, USA) formulation of tulathromycin 2.5 mg injection after intramuscular administration of a single dose. Twelve pigs were randomly allocated to two treatment groups. Blood samples were collected by venipuncture of the jugular vein or anterior vena cava, plasma samples were analyzed by High-Performance Liquid Chromatography (HPLC) with tandem mass spectrometry detection (LC-MS/MS) using ESI. Mean puls or minus Standard Deviation (SD) of peak plasma Concentration (C_{max}) Area Under the serum Concentration-time curve (AUC_{0-t}), Area Under the serum Concentration-time curve (AUC_{inf}), serum concentration half-life ($t_{1/2}$) were 4.32±1.52 and 5.86±1.28 μg mL^{-1}, 3.98±1.63 and 4.24±1.30 μgh mL^{-1}, 4.04±1.67 and 4.65±2.01 μgh mL^{-1}, 83.55±12.84 and 79.25±10.64 h for the locally manufactured (tested) and reference formulation, respectively. The 90% confidence intervals of the mean of the difference between log-transformed values for AUC_{0-360}, $AUC_{0-\infty}$ and C_{max} were within the bioequivalence accepted range of 80-125%. The results indicate that tulathromycin was rapidly absorbed, eliminated slowly and highly bioavailable following a single dose which make tulathromycin likely to be effective in swine.

Key words: Tulathromycin, pharmacokinetic, bioavailability, bioequivalence, swine, China

INTRODUCTION

Actinobacillus pleuropneumoniae is one of the principal etiologic agents of contagious bacterial disease in swine which causes pleuropneumoniae (Nicolet, 1992; Taylor, 1999). Tulathromycin is a broad-spectrum antibiotic with *in vitro* activity against certain Gram-negative and Gram-positive bacterial pathogens including the bacterial pathogens most commonly associated with Swine Respiratory Disease (SRD) and Bovine Respiratory Disease (BRD). According to the relevant reports, tulathromycin can also be used to prevent severe pneumonia in goats and foals. In veterinary medicine macrolides are normally administered by deep IM injection (Angen *et al*., 2008; Burrows *et al*., 1989; Benchaoui *et al*., 2004).

Tulathromycin is a newly introduced triamilide antimicrobial labeled for the treatment of respiratory disease in cattle and swine. Because of tulathromycin's efficacy in cattle against the same bacteria commonly isolated from pigs (Godinho *et al*., 2005; McKelvie *et al*., 2005; Hellman *et al*., 2008; Icen *et al*., 2009; Ragbetli *et al*., 2009; Skogerboe *et al*., 2005), its efficacy against Mycoplasma and its long-acting properties (Nowakowski *et al*., 2004), it may provide an effective tool in the treatment of caprine respiratory disease. The safety of tulathromycin administration in pigs and cattle were studied; physical and laboratory parameters between treatment and control groups prior to treatment yielded no significant differences (Washburn *et al*., 2007). The pharmacokinetics of tulathromycin following subcutaneous administration in meat goats were also researched (Young *et al*., 2011).

The Pharmacokinetics (PK) of tulathromycin has been studied in pre-ruminant and ruminant cattle and in pigs, the pharmacokinetic profile of tulathromycin administered

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as a single injection at the proposed label dose (2.5 mg kg^{-1} BW, single subcutaneous dose in cattle, intramuscularly in pigs) is characterized by rapid and extensive absorption followed by high distribution and slow elimination.

The objective of this study was to compare the bioequivalence and pharmacokinetics of two injection formulations of tulathromycin in healthy swine after a single dose IM administration.

MATERIALS AND METHODS

Treatments: Each treated animal received a single injection of tulathromycin, a locally manufactured (tested) formulation (Tulathromycin injection, CONTINENT, China) and a prototype commercial injectable formulation (DRAXXIN, Pfizer Animal Health, USA). Intramuscular (IM) injections were made into the cervical muscles with an 18G 40 mm needle. Treatments were administered using a 1 mL syringe and the actual dose administered was calculated by weighing each syringe before and after injection.

Animals: About 12 clinically healthy Landrace x Large white pigs (age range 2-3 months; weight range 23.0-25.7 kg) with a history of no macrolide administration were enrolled in the studies. Pigs in each study were purchased from a single commercial supplier, identified by numbered ear tag and acclimatized for at least 7 days prior to administration of treatment on day 0. Animals were observed daily for general health and clinical observations were made prior injection and at 1-2, 4-5 and 24 h postinjection. The studies were designed and conducted in accordance with Good Laboratory Practice standards and National Welfare regulations.

Experiment design: Twelve pigs were randomly allocated to two treatment groups (TB1 and TB2). Each group contained six animals. Tulathromycin was administered via the IM route to all animals. The timing of blood collection was plannaed according to the preciously reported value of time to reach peak serum concentration (T_{max}) and serum elimination harf-life ($t_{1/2}$). Blood samples were collected predose and at 5, 10, 15, 30, 45 min and 1, 2, 4, 8, 16, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, 336, 360 h after drug administration.

Sample collection and determination of tulathromycin level: Blood samples were collected by venipuncture of the jugular vein. The blood was transferred into heparinized tubes; the plasma was harvested and stored frozen (approximately -20°C) for later analysis.

Plasma samples were analysed by High-Performance Liquid Chromatography (HPLC) with tandem mass spectrometry detection (LC-MS/MS) following protein precipitation. Plasma samples were analysed singly and roxithromycin was used as Internal Standard (IS).

Tulathromycin was extracted from plasma by adding 0.2 mL of working internal standard solution to 0.5 mL of plasma in a plastic tube; this gave a plasma internal standard concentration of 200 μg L^{-1}. Samples were vortexed and then centrifuged (at 1700 g for 5 min) before being applied to a cation exchange cartridge (MCX, 500 mg, 3 m). The SPE cartridge had been earlier washed with 2 mL acetonitrile and 2 mL phosphate buffer (pH 6.8). The sample was washed with 2 mL phosphate buffer and 2 mL acetonitrile.

The extracted samples were analysed using a Themo Hypersil Gold 5 μm C18 column (150×2.1 mm) at a flow rate of 0.25 mL min^{-1}. Solvent A consisted of 2 mM ammonium acetate and formic acid (999:1, v/v) and solvent B was acetonitrile. Mass spectrometric detection was conducted on Thermo TSQ Quantum mass spectrometer (Thermo, USA) equipped with an Electrospray Ionization ion Source (ESI) interface operated in the positive ionization mode. All the parameters of HPLC and MS/MS were controlled by the device-specific software Quantum Tme Maste (Thermo, USA).

Linearity of the calibration curve for plasma was shown between 2.0 and 1600 ng mL^{-1}. The linear calibration range of the method was used for plasma analysis by increasing the IS concentration up to 200 ng mL^{-1}. The concentration values of 2 and 5 ng mL^{-1} were set as the LOD and LOQ of the method, respectively. The precision and accuracy were investigated with Quality Control (QC) samples at concentrations of 5,125 and 1600 ng mL^{-1}. Results are shown in Table 1. Over the validated range the within and between-batch accuracy for plasma was in the range 1.11-4.95% and 6.34-6.78%, respectively.

Pharmacokinetic and statistical analysis: Noncompartetal pharmacokinetic analysis was performed with the serum concentration-time profile of individual animals using the WinNonlin™ Software package (Standard edition Version 6.1, Pharsight Corporation, CA,

Table 1: Precision and accuracy of the method for determing Ciprofloxacin levels in swine plasma (n = 5)

Concentration (ng mL^{-1})			(RSD %)	
Added	Found	Relative error* (%)	Intra-day	Inter-day
5	5.25	4.54	4.95	6.78
125	128.02	6.42	4.05	9.72
1600	1569.87	-1.88	3.12	6.34

*Relative error = (mean, easured concentration-added concentration)× 100/added concentration. RSD = Relative Standard Deviation

USA). WinNonlin™ Model 200 (for IM) was used for the noncompartmental analysis of the time and concentration data.

Statistical analyses were performed using Systat Software (Version 16.0; SPSS Inc., Chicago, IL, USA). The arithmetic mean and standard deviation were calculated for all parameters excepted for half-life values where harmonic mean values and standard deviation were calculated according to Lam *et al*. (1985). The parameters were compared using a paired t-test. A level of significance was set at 0.05.

RESULTS AND DISCUSSION

The mean (±SD) tulathromycin plasma concentration-time profiles following IM administration are shown in Fig. 1 was similar and superimposable. The plasma disposition of tulathromycin after IM administration at 2.5 mg kg^{-1} BW could be described by the Noncompartmental Model.

Central and dispersion measures for all pharmacokinetic parameters for both formulations are shown in Table 2. Following IM administration of 2.5 mg kg^{-1} BW, peak plasma concentrations (Cmax 5.86±1.28 μg mL^{-1}) were reached at 0.25±0.00 h for the reference product and 4.32±1.52 μg mL^{-1}) were reached at 0.25±0.00 h for the locally manufactured (test) product. For the Area Under the Curve (AUC_{0-inf}) was 4.65±2.01 and 4.04±1.67 hμg mL^{-1}, the Mean Residence Time (MRT) was 92.35±17.46 and 98.76±18.18 h and the terminal half-life ($t_{1/2}$) was 79.25±10.64 and 83.55±12.84 h, respectively.

The Cmax, tmax, AUC, MRT, Vss, Cl and terminal half-life ($t_{1/2}$) did not differ significantly with dosage (Table 3) following intramuscular (IM) administration to pigs at 2.5 mg kg^{-1} body weight.

Assessment of bioequivalence of local product to reference product is required to exclude any clinically important differences in the rate or extent at which the active entity of becomes available at the site of action. Two drugs are considered to be bioequivalent if they are pharmacokinetically equivalent and their bioavailability is so similar that they are unlikely to produce clinically relevent differences in regard to safety and efficacy.

The aim of this study was to compare the bioavailability of 2 formulations of tulathromycin 5% injection, a locally manufactured (test) formulation and a reference formulation, Draxxin. The study revealed that at a 90% confidence interval (Table 4) AUC0-t, AUCinf and Cmax were found to be 98.45 and 102.98%; 97.24 and 102.35% and 98.62 and 102.76%, respectively, from log-transformed date and all values are within the bioequivalence accepted range of 80-125%. Moreover, a further evaluation of the rate of absorption was performed

Table 2: Mean Pharmacokinetic parameters of Tulathromycin. (Reference formulation and Tulathromycin (tested))

Pharmacokinetic parameters	Reference formulation	Tulathromycin (tested)
C_{max} (μg mL^{-1})	5.86±1.280	4.32±1.5200
t_{max} (h)	0.25±0.000	0.25±0.0000
AUC_{0-t} (hμg mL^{-1})	4.24±1.300	3.98±1.6300
AUC_{inf} (hμg mL^{-1})	4.65±2.010	4.04±1.6700
$AUMC_{inf}$ (hhμg mL^{-1})	370.25±98.54	361.55±104.95
MRT (h)	92.35±17.46	98.76±18.180
Vss (L kg^{-1})	64.28±18.25	62.18±20.290
Cl (L/h/kg)	0.61±0.210	0.53±0.1900
$t_{1/2}$ (h)	79.25±10.64	83.55±12.840

C_{max} = Maximum plasma Concentration; t_{max} = Time to reach peak serum concentration. AUC_{0-t} = Area Under the plasma Concentration-time curve from time 0 to the last quantifiable timepoint (tlast); AUC_{inf} = The Area Under the serum Concentration-time curve extraplolated to infinity; AUMC = Area Under the first Monment versus time Curve from 0 to infinity; MRT = Mean Resident Time; Vss = Volume of distribution at steady-state; Cl = Plasma Clearance; $t_{1/2}$ = Mean elimination half-life (apparent $t_{1/2}$ for IM route)

Table 3: p-values for different Pharmacokinetic parameters of 2 formulations calculated by paired t-test

Pharmacokinetic	AUC_{0-t}	AUC_{inf}	C_{max}	t_{max}	$t_{1/2}$	MRT	AUMC	Cl
p-values	0.624	0.784	0.615	0.65	0.71	0.65	0.66	0.53

AUC_{0-t} = Area Under the plasma Concentration-time curve from time 0 to the last quantifiable timepoint (tlast); AUC_{inf} = The Area Under the serum Concentration-time curve extraplolated to infinity; C_{max} = Maximum plasma Concentration; t_{max} = Time to reach peak serum concentration. $t_{1/2}$ = Mean elimination half-life (apparent $t_{1/2}$ for IM route); MRT = Mean Resident Time; AUMC = Area Under the first Monment versus time Curve from 0 to infinity; Cl = Plasma Clearance

Table 4: Large sample-based 90% Confidence Intervals (Cl) for different Pharmacokinetic Parameters from log-transformed and untransformed date for assessment of bioequivalence

	Test (Tulathromycin Injection)/Reference (Draxxin)					
	Untransformed			Log transformed		
Parameters	Mean ratio (Test/Reference) (%)	90% Cl		Mean ratio (Test/Reference) (%)	90% Cl	
AUC0-t (hμg mL^{-1})	102.34	87.57%	118.21%	101.24	98.45%	102.98%
AUC_{inf} (hμg mL^{-1})	98.84	86.54%	113.55%	100.04	97.24%	102.35%
C_{max} (μg mL^{-1})	97.98	96.41%	101.30%	100.85	98.62%	102.76%
C_{max}/AUC_{inf}	119.42	120.14%	121.16%	103.21	98.25%	105.64%

AUC_{0-t} = Area Under the plasma Concentration-time curve from time 0 to the last quantifiable timepoint (tlast); AUC_{inf} = The Area under the serum Concentration-time curve extraplolated to infinity; C_{max} = Maximum plasma Concentration; Cl = Confidence Interval

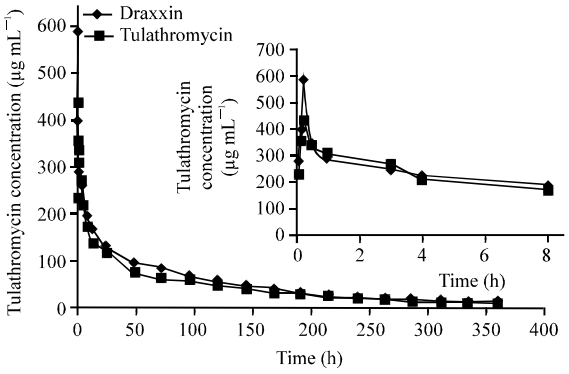

Fig. 1: Mean plasma concentrations of tulathromycin at different time intervals after single intramuscular administration of 5% of tulathromycin injection to 12 pigs

by analyzing the C_{max}/AUC_{inf} since this parameter has been proposed to better reflect the absorption rate. The 90% confidence intervals for this parameter also indicated bioequivalence.

CONCLUSION

In this study, the two formulations can be considered bioequivalent in regard to the extent and rate of absorption and therefore interchangeable.

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