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Xu Wang
Huazhong Agricultural University

Yanfei Tao
Wageningen University

Awais Ihsan
COMSATS Institute of Information Technol…

Zhen-Hong Yuan
Chinese Academy of Sciences

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Pharmacokinetics of tulathromycin and its metabolite in swine administered with an intravenous bolus injection and a single gavage

X. WANG*
Y. F. TAO*
L. L. HUANG*
D. M. CHEN*
S. Z. YIN†
A. IHSAN*
W. ZHOU*
S. J. SU*
Z. L. LIU*
Y. H. PAN* &
Z. H. YUAN*

**National Reference Laboratory of Veterinary Drug Residues and MOA Key Laboratory of Food Safety Evaluation, Huazhong Agricultual University, Wuhan, China; †Qingdao Continent Pharmaceutical Co., Ltd, Qingdao, China*

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Tulathromycin is a macrolide antimicrobial agent proposed for therapeutic use in treatment of porcine and bovine respiratory disease. In this study, the absolute bioavailability of tulathromycin solution was investigated in pigs. Eight pigs, with body weight of 20.5 ± 1.6 kg, were given a single dose of tulathromycin at 2.5 mg/kg oral (p.o.) and intravenous (i.v.) in a crossover design. The plasma concentrations of tulathromycin and its metabolite were determined by LC–MS/MS method, and the pharmacokinetic parameters of tulathromycin were calculated by noncompartmental analysis. After p.o. administration, the maximum plasma concentration (C_{max}) was 0.20 ± 0.05 μg/mL at 3.75 ± 0.71 h. The terminal half-life ($t_{1/2\lambda z}$) in plasma was 78.7 ± 6.75 h, and plasma clearance (*Cl*/F) was 1.14 ± 0.28 L/h/kg. After i.v. injection, plasma clearance (*Cl*) was 0.580 ± 0.170 L/h/kg, the volume of distribution (*Vz*) was 64.3 ± 21.2 L/kg, and the $t_{1/2\lambda z}$ was 76.5 ± 13.4 h. In conclusion, an analytical method for the quantification of tulathromycin and its metabolite in plasma in swine was developed and validated. Following p.o. administration to pigs at 2.5 mg/kg b.w., tulathromycin was rapidly absorbed and the systemic bioavailability was 51.1 ± 10.2.

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Professor Zonghui Yuan, National Reference Laboratory of Veterinary Drug Residues (HZAU)/MOA Key Laboratory of Food Safety Evaluation, Huazhong Agricultural University, Wuhan, Hubei 430070, China. E-mail: yuan5802@mail.hzau.edu.cn

INTRODUCTION

Tulathromycin ($C_{41}H_{79}N_3O_{12}$; *M*: 806.1 g/mol) is a novel semi-synthetic macrolide antibiotic of the triamilide group. It is approved for the treatment of bacterial respiratory diseases in swine and cattle in Europe and the USA in 2004 (Benchaoui *et al.*, 2004). The injectable solution of tulathromycin is indicated for the treatment of bovine respiratory disease (BRD) and swine respiratory disease (SRD), such as *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, and *Bordetella bronchiseptica*. As major contagious respiratory diseases in pigs, porcine pleuropneumonia and porcine pneumonia are distributed worldwide and cause high morbidity with severe economic losses to the swine industry (Sidibe *et al.*, 1993; Pijoan, 1999; Dziva *et al.*, 2008). Recently, tulathromycin was also used in the treatment of abscessing pneumonia of foals caused by *Rhodococcus equi* (Scheuch *et al.*, 2007).

Other macrolide antibacterial agents, such as tilmicosin, erythromycin, spiramycin, and tylosin, are in the form of drinking water or feed premixes for control, prevention or treatment of bacterial respiratory disease in pigs (Benchaoui *et al.*, 2004). To achieve therapeutic or preventative success, these drugs are required to be administrated repeatedly over several days. Injectable tulathromycin has been identified to be effective when used only once in clinical practice. After intramuscular (i.m.) injections, tulathromycin is nearly completely absorbed from the injection site to reach maximal serum concentrations within half an hour (Benchaoui *et al.*, 2004). The systemic bioavailability following i.m. administration is >87%, and tulathromycin is widely distributed and accumulates in lung tissue of swine (Benchaoui *et al.*, 2004; Galer *et al.*, 2004). Commonly, it was preferable for veterinarians to use injectable antibiotics for the treatment of porcine and bovine pneumonia and pleuropneumonia. Using injections, the

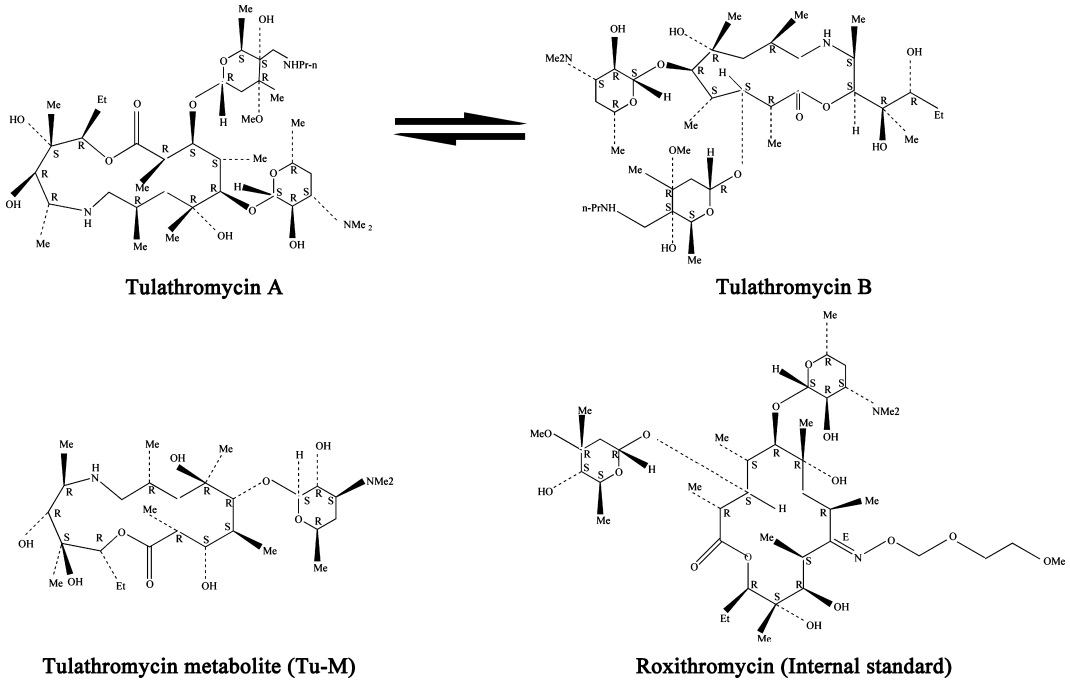

Fig. 1. The chemical structures of tulathromycin A, tulathromycin B, metabolite of tulathromycin and roxithromycin.

veterinarian has better control over the medication regimen, and the animals can receive the appropriate dosage. However, individual handling and treatment of the animals will cause additional stress to the animals in certain epidemiological situations. Oral treatment will be easier and less laborious in the case of a herd outbreak. Tulathromycin solution may be in the form of drinking water and offers an alternative choice of administration route for the animal treatment. It is particularly convenient for the treatment of a big group of animals. Therefore, it is meaningful to perform pharmacokinetic studies in the target species to obtain information about the behaviors of different tulathromycin formulations, such as oral formulation.

Tulathromycin is consisted of a regioisomeric, equilibrated mixture of 13-member ring azalide (isomer B, 10%) and 15-member ring azalide (isomer A, 90%), both with three basic amine groups (Galer *et al.*, 2004; Fig. 1). In previous studies, the validation of quantitative assays was focused on isomer A because equilibrium in aqueous solutions was reached within 48 h (Benchaoui *et al.*, 2004; Galer *et al.*, 2004; Nowakowski *et al.*, 2004). Therefore, isomer A was quantitatively analyzed in this study. Metabolic elimination via N-oxidation and N-demethylation is only of minor significance (Scheuch *et al.*, 2007). Tulathromycin may be hydrolyzed to CP- (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R) -2-ethyl-3,4,10,13-tetrahydroxy-3,5,8,10,12,14-hexamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one (Tu-M) (European Medicines Agency Veterinary Medicines and Inspections, EMEA, 2002; Fig. 1). The detection of the Tu-M in plasma may contribute to understand the disposition kinetics of Tu-M in pigs.

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was used to determine tulathromycin in plasma (Benchaoui *et al.*, 2004; Galer *et al.*, 2004; Nowakowski *et al.*, 2004; Scheuch *et al.*, 2007), lung (Benchaoui *et al.*, 2004; Galer *et al.*, 2004; Nowakowski *et al.*, 2004), and muscle (Martos *et al.*, 2008). None of the above-mentioned methods can be used to analyze tulathromycin and its metabolite simultaneously. In this study, we firstly built a LC–MS/MS method for quantification of tulathromycin and its metabolite in pig plasma. Our LC–MS/MS method was based on the internal standard method and did not use radiolabeled reference compounds.

To study the bioavailability of tulathromycin solution and disposition kinetics of its metabolite, we established a selective LC–MS/MS method to determine the concentration of tulathromycin and its metabolite in pig plasma. We then investigated the pharmacokinetics of tulathromycin and its metabolite in pigs following single oral and intravenous administrations. Furthermore, we discuss the comparative pharmacokinetics of tulathromycin with other species and different administration routes.

MATERIALS AND METHODS

Drugs and reagents

Tulathromycin ($C_{41}H_{79}N_3O_{12}$, 806.1 g/mol, CAS: 217500-96-4, >95% purity), metabolite of tulathromycin (Tu-M, $C_{29}H_{56}N_2O_9$, 577 g/mol, CAS: 111247-94-0, >95% purity) and 100 mg/mL tulathromycin solution were purchased from Qingdao Liuhe Pharmaceutical Co., Ltd. (Qingdao, China). Roxithromycin ($C_{41}H_{76}N_2O_{15}$, 837.05 g/mol, CAS: 80214-83-1, >94% purity)

was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Peking, China). The chemical structures of tulathromycin, its metabolite, and roxithromycin are shown in Fig. 1. HPLC-grade formic acid was purchased from Tedia Company Inc (Fairfield, OH, USA). Methanol and methyl cyanides were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China), and they were all of HPLC grade. Purified water was generated by a Millpore Q reagent water system (Billerica, MA, USA). All other reagents were also of analytical grade.

Animals

Eight healthy 2-month-old Duroc × landrace × Youkshire crossbred castrate piglets, weighing 20.5 ± 1.6 kg at the start of the experiments, were used in the study. Pigs enrolled in the studies had no history of macrolide administration. Pigs were purchased from Huazhong Agricultural Pig Farm (Wuhan, China). The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine (Huazhong Agricultural University).

Animals were housed separately per pen, with a minimum floor area of 3 m^2 per animal. The animal house was maintained at 20–28 °C and 40–65% relative humidity with a 12:12 h light/dark cycle. The pigs were supplied with a drug-free commercial diet. Each pig was identified by numbered ear tag and acclimatized for at least 7 days prior to administration. Pigs were observed daily for general health, and clinical observations were made before and after injection. All diets were manufactured by Wuhan Tianyou Feed Co. Ltd (Wuhan, China) and were formulated to meet or exceed the requirements described by the NRC (1998). No antimicrobial was supplemented in the experimental diets. Food and water were supplied *ad libitum*. Use of animals in this study was in accordance with Good Laboratory Practice standards and national welfare regulations.

Administration to animals

A 100 mg/mL tulathromycin solution was diluted to 25 mg/mL (for intravenous injection) and 2.5 mg/mL (for oral administration) with the double-distilled water containing a 50% propylene glycol vehicle and monothioglycerol (5 mg/mL). The 1 mL of solution contains 25 or 2.5 mg tulathromycin with pH value of 7.0, which was adjusted with citric and hydrochloric under sterile conditions prior to drug administration. The pigs were randomly divided into two groups. Pigs of group I (4 pigs) were administered by a single i.v. bolus injection in the ear vein at a dose of 2.5 mg/kg b.w. Pigs of group II (4 pigs) were administered with the same dose tulathromycin orally directly into the stomach using a thin plastic tube attached to a syringe. Except water, pigs were not fed for 12 h before treatments until 4 h after drug administration. After a 20-day washout interval, pigs of group I were administered with 2.5 mg/kg b.w. tulathromycin orally and pigs of group II were administered by a single i.v. bolus injection in the ear vein at a dose of 2.5 mg/kg b.w. tulathromycin.

Collection of plasma samples

Blood samples (each of 2.0 mL) were taken from the precaval vein at the times 0.0, 0.08, 0.17, 0.33, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, and 216 h after drug administration. Blood samples were immediately transferred into heparinized tubes and separated by centrifugation (Sigma 3K15, Osterode am Harz, Germany) at 1000 ***g*** for 10 min. The plasma was harvested and stored frozen (−70 °C) until assayed. The concentrations of tulathromycin and Tu-M were determined by LC–MS/MS.

Sample preparations for LC–MS/MS analysis

About 500 μL plasma was measured into a 10-mL polypropylene centrifuge tube. Then, 200 μL roxithromycin (0.20 μg/mL) was added as internal standard. The mixture was vortexed for 1 min. After vigorous shaking, followed by centrifugation at 5000 ***g*** for 10 min at 4 °C, the supernatant was applied to a cation-exchange cartridge (Waters Oasis MCX, 3cc; Waters Corporation, Milford, MA, USA). The SPE cartridge had been previously washed with 2 mL acetonitrile and 2 mL phosphate buffer (pH 6.8). The phosphate buffer comprised 0.354% sodium dihydrogen phosphate and 0.277% disodium hydrogen phosphate. After being washed with 1.0 mL phosphate buffer, the sample was washed with 2 mL acetonitrile and then washed twice with 2 mL acetonitrile/ammonia (95:5, v/v). The eluates were evaporated to dryness at 50 °C using N_2 with water bath. The residue was reconstituted in 200 μL solution [50% methanol A–50% mobile phase B (0.1% formic acid–0.032% ammonium formate aqueous solution), v/v] followed by centrifugation at 15 000 ***g*** for 20 min at 4 °C and transferred 120 μL supernatant to autosampler vials for LC–MS/MS analysis. When samples had concentrations of tulathromycin >800 ng/mL for plasma, aliquots of the samples were diluted with control swine plasma before analysis, and the data were corrected for the dilution factor.

LC–MS/MS conditions

Finnigan HPLC equipped with a quaternary gradient surveyor LC pump and a surveyor as autosampler MS/MS analyses (Thermo Fisher. Scientific, Waltham, MA, USA) was performed on a TSQ Quantum Access triple quadrupole mass spectrometer (Thermo Finnigan, San Jose, CA, USA). The system was controlled by Xcalibur software (Thermo Finnigan).

A Hypersil Gold C-18 column (5 μm, 2.1 × 150 mm; Thermo Finnigan) was used for the separation of tulathromycin, its metabolite and roxithromycin from plasma extracts. The mobile phase consisted of A (methanol) and B (0.1% formic acid–0.032% ammonium formate aqueous solution) with gradient elution (Table 1). The flow rate was 0.25 mL/min. The column was maintained at 46 °C, and the injection volume was 10 μL. Electrospray ionization (ESI) source was applied and operated in the positive ion mode. The spray voltage was set at 4500 V. Sheath gas pressure and Aux gas pressure were set at 10 and

Table 1. Mobile phase gradient elution program

Time (min)	Mobile phase A	Mobile phase B
0.00	15.00	85.00
1.00	15.00	85.00
5.00	90.00	10.00
10.00	90.00	10.00
10.10	15.00	85.00
20.00	15.00	85.00

5 arb, respectively. The capillary temperature was 300 °C. High-purity argon gas was used as collision gas at a pressure of 0.2–0.3 MPa for collision-induced dissociation (CID). Selected reaction monitoring (SRM) mode was used to quantify drugs at *m/z* 577.14 → 115.81 and 577.14 → 420.09 for Tu-M, *m/z* 806.60 → 576.72 and 806.60 → 158.30 for tulathromycin, and *m/z* 837.58 → 158.18 and 837.58 → 679.33 for roxithromycin, respectively. The optimized collision energies of 35 and 21 eV were used for tulathromycin, 28 and 22 eV were used for Tu-M, and 30 and 16 eV were used for roxithromycin.

Preparation of calibration standards and quality control samples

Standard stock solutions of tulathromycin, Tu-M, and roxithromycin were prepared individually in methanol at the concentrations of 1000 μg/mL for analyte. Standard stock solution was further diluted with mobile phase to achieve standard working solutions at concentrations of 0.005, 0.025, 0.05, 0.125, 0.4, 0.8, and 1.6 μg/mL. Quality control samples were also prepared in the same way, using a separately weighed stock solution. All the working solutions were prepared before use and kept at 4 °C.

Plasma samples from pigs for calibration curve were prepared by spiking different 500 μL blank plasmas with the appropriate volumes of the mixed working solutions of tulathromycin and Tu-M, to produce the calibration standards at concentrations of 0.005, 0.025, 0.125, 0.4, and 0.8 μg/mL. Quality control (QC) samples were prepared at three different concentration levels: low level (0.005 μg/mL), middle level (0.125 μg/mL) and high level (0.8 μg/mL). The plasma internal standard concentration was 0.2 μg/mL.

The chromatograms were evaluated with the internal standard method using peak-area ratios for calculation. The definition of LOD (S/N = 3) of each analyte was adopted when the ion giving the worst S/N was considered in each case.

Pharmacokinetic analysis

Pharmacokinetic parameters were determined using Winnonlin, version 5.0.1 (Pharsight, Mountain View, CA, USA). WinNonlin™ model 200 for p.o. administration and model 201 for i.v. administration were used for the noncompartmental analysis of the time and concentration data. The linear trapezoidal approximation was used to calculate the AUC_{last}, which was under the plasma concentration–time curve from time 0 to the last time point t_{last} with a measurable concentration (C_{last}). The $C_{est}/\lambda z$ was estimated from t_{last} to infinity and summed with AUC_{last} to give an estimate of total AUC_{inf}, where C_{est} represented the estimated concentration at the last time point, and λz was the first-order rate constant associated with the terminal (log linear) elimination phase. During the terminal log-linear elimination phase, λz was calculated using least squares regression analysis of the concentration–time data obtained. The terminal half-life ($t_{1/2\lambda z}$) was calculated as ln 2/λz using harmonic mean values. The maximum plasma concentration (C_{max}) was represented directly from the data with t_{max} defined as the time of the first occurrence of C_{max}. The mean residence time (*MRT*), the total body clearance (*Cl*), and volume of distribution at steady state (V_{ss}) were also determined. The bioavailability (*F*) was calculated as follows:

$$F(\%) = \frac{\text{Least squares mean } AUC_{last}(\text{p.o. route})}{\text{Least squares mean } AUC_{last}(\text{i.v. route})} \times 100. \quad (1)$$

In this study, the absolute bioavailability (*F*) of tulathromycin was calculated.

RESULTS

LC–MS/MS analysis of tulathromycin, Tu-M, and roxithromycin

By injecting standard solutions into a mass spectrometer using an electrospray ionization source, the precursor ions for tulathromycin, Tu-M, and roxithromycin were identified from spectra obtained. The parameters for fragmentor energies and collision energies were optimized to obtain the richest relative abundance of precursor ions and product ions, and the SRM transition was chosen at *m/z* 806.60 → 576.72 and 806.60 → 158.30 for tulathromycin, *m/z* 577.14 → 115.81 and 577.14 → 420.09 for Tu-M, and *m/z* 837.58 → 158.18 and 837.58 → 679.33 for roxithromycin. The corresponding product ion scan spectra are shown in Fig. 2.

Validation of the LC–MS/MS method to determine tulathromycin, Tu-M, and roxithromycin in plasma samples

The representative chromatograms of tulathromycin, Tu-M, and roxithromycin in plasma are shown in Fig. 3, respectively. The retention times of tulathromycin, Tu-M, and roxithromycin were 9.06, 8.56, and 11.18 min, respectively. There was good baseline separation of them from each other and from the chromatographic background noise. No endogenous or extraneous peaks were observed interfering with the assay. The method exhibited a good linear response for the concentration range of 0.005–1.6 μg/mL. The typical linear equation was $y = 0.0008x + 0.0086$ ($r = 0.9995$) and $y = 0.0003x + 0.0049$ ($r = 0.9988$) for the standard solution of tulathromycin and Tu-M, respectively. The calibration graphs of spiked plasma samples were plotted in the concentration range 0.005–0.8 μg/mL. The typical linear equations were $y = 0.0009x - 0.0055$ ($r = 0.9998$) and $y = 0.0004x - 0.0044$ ($r = 0.9952$) for tulathromycin and Tu-M, respectively. LOQ of the method was found to be 0.005 μg/mL for both tulathromycin and Tu-M

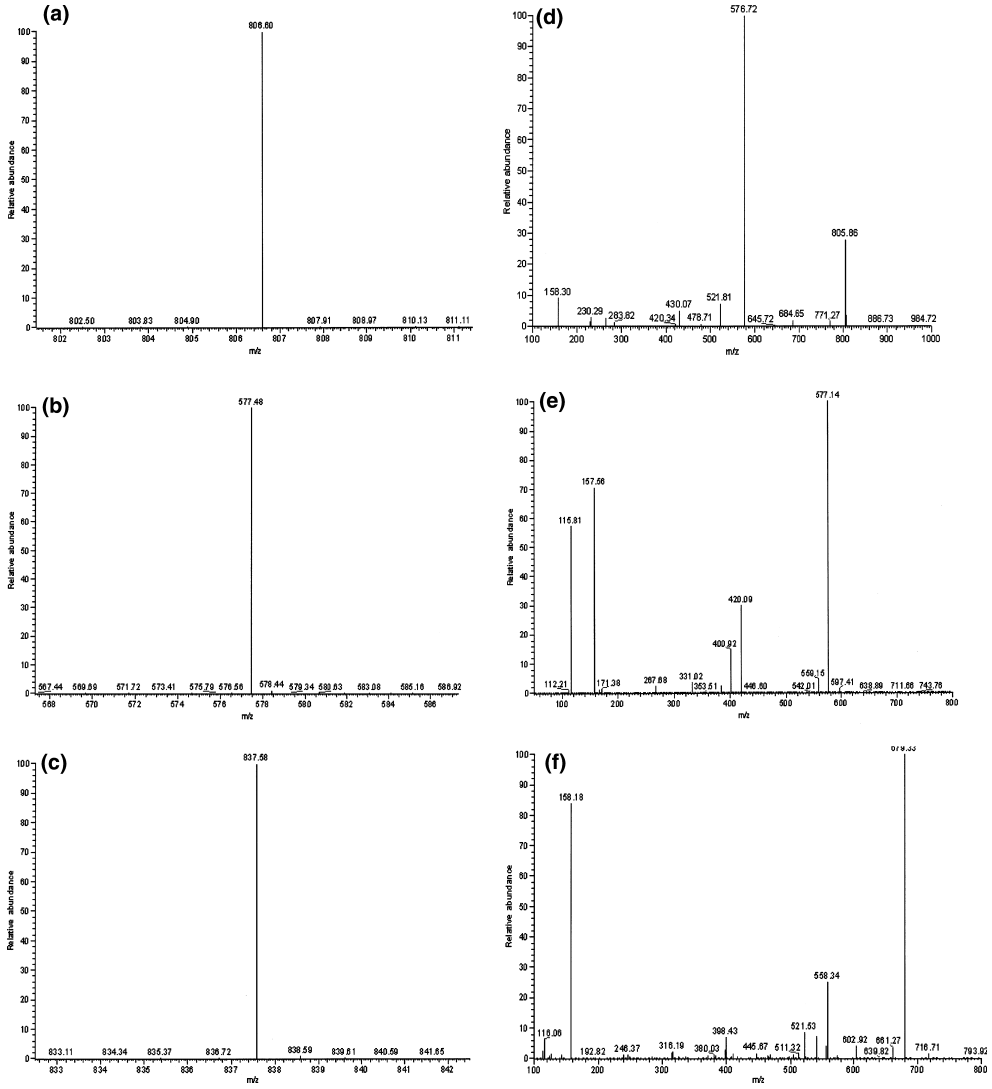

Fig. 2. Positive ion electrospray mass spectra of (a) tulathromycin, (b) metabolite of tulathromycin, and (c) roxithromycin. The product ions were monitored at m/z 577.14 → 115.81 and 577.14 → 420.09 for metabolite of tulathromycin (d), m/z 806.60 → 576.72 and 806.60 → 158.30 for tulathromycin (e), and m/z 837.58 → 158.18 and 837.58 → 679.33 for roxithromycin (f).

(Fig. 3), which was sufficient for pharmacokinetics and clinical studies of them. The LOD was 0.002 and 0.001 μg/mL for tulathromycin and Tu-M, respectively. Within-day accuracy and within-day and between-day precision for plasma samples were determined by assaying plasma spiked with various concentrations of tulathromycin and Tu-M. Five replicates were assayed at each of three levels, and these results are shown in Table 2. Recoveries averaged were within the range of 86% to 103% at concentration levels of 0.005, 0.125, and 0.8 μg/mL with a coefficient of variation range between 0.2% and 10%.

Clinical observations and pharmacokinetics of tulathromycin

All pigs were clinically healthy throughout the experimental period. No adverse cardiovascular effects associated with i.v. administration of some other macrolide compounds, e.g. tilmicosin (Ziv *et al.*, 1995; Modric *et al.*, 1998), were observed following p.o. and i.v. administrations of tulathromycin in pigs. Therefore, the bioavailability of tulathromycin solution administered orally could be determined in this study. Because the concentrations of Tu-M (5–8 ng/mL) were very low in this study, the pharmacokinetic analysis was focused on tulathromycin. The pharmacokinetic parameters of tulathromycin (mean ± SD) based on noncompartmental pharmacokinetic analysis are shown in Table 3. After a single i.v. of tulathromycin (2.5 mg/kg), the plasma tulathromycin concentration reached maximum serum concentrations (C_{max}) of 5.43 ± 2.71 μg/mL at 0.08 h. The mean volumes of distribution (V_z) were 64.3 ± 21.2 L/kg, and the mean terminal half-lives ($t_{1/2\lambda z}$) were 76.5 ± 13.4 h. After a single oral administration of tulathromycin (2.5 mg/kg), tulathromycin reached C_{max} of 0.200 ± 0.0500 μg/mL within about 2–4 h. The mean

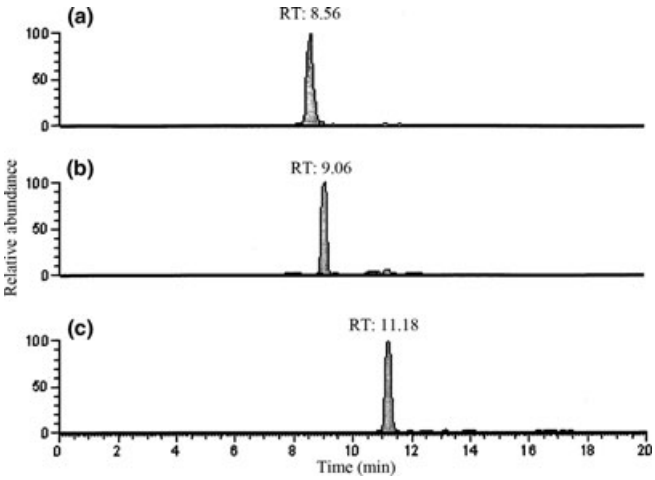

Fig. 3. Selective reaction monitoring chromatograms of pig plasma sample spiked with (a) metabolite of tulathromycin, (b) tulathromycin, and (c) roxithromycin. The product ions were monitored at *m/z* 577.14 → 115.81 and 577.14 → 420.09 for metabolite of tulathromycin, *m/z* 806.60 → 576.72 and 806.60 → 158.30 for tulathromycin, *m/z* 837.58 → 158.18 and 837.58 → 679.33 for roxithromycin.

volumes of distribution were 130 ± 32.3 L/kg, and the mean terminal half-lives were 78.7 ± 6.75 h. The systemic bioavailability of tulathromycin was 51.1 ± 10.2%. The plasma pharmacokinetic parameters after a single intravenous injection and an oral administration are shown in Table 3, and a semi-logarithmic plot of the concentration–time curve for plasma is shown in Fig. 4.

DISCUSSION

Tulathromycin is commonly used in swine for respiratory diseases caused by *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Pasturella multocida*, and *Mycoplasma hyopneumoniae* and in cattle for bovine respiratory disease caused by *Pasturella multocida* and *Mannheimia haemolytica* (Nutsch *et al.*, 2005). To *Pasteurella multocida*, it was previously reported that MIC_{90} for tulathromycin was 2 μg/mL in 2004–2005 and 1 μg/mL in 2005–2006 (Kaspar *et al.*, 2007). To *Actinobacillus pleuropneumoniae*, the MIC_{90} for tulathromycin was 16 μg/mL (Benchaoui *et al.*, 2004). Compared with the high MIC_{90}, the plasma of tulathromycin after i.v. or i.m. appeared to be low (Benchaoui *et al.*, 2004). Because lung is an important organ harmed in the progresses of SRD and BRD, higher concentration of drug in lung may result in good antibacterial effect. High distribution into lung was found for macrolide drugs, such as azithromycin, clarithromycin, and tulathromycin (Rodvold *et al.*, 1997; Zhanel *et al.*, 2001; Benchaoui *et al.*, 2004; Galer *et al.*, 2004). Tulathromycin concentrations in the lung were 24.9–181 times higher than those measured in plasma (Benchaoui *et al.*, 2004; Galer *et al.*, 2004), which was interpreted as one important reason for good clinical efficacy (Benchaoui *et al.*, 2004). Therefore, we presumed that the tulathromycin concentrations in the lung were much higher than those measured in plasma after oral administration. And the presumption should be identified by the later test. As for the higher concentration of tulathromycin in lung than in plasma, factors such as bulk flow because of pressure gradient, active transport, and permeation through nonporous membranes were thought to play important roles (Chiu & Amsden, 2002; Benchaoui *et al.*, 2004). However, it is difficult to predict the tulathromycin antibacterial efficacy *in vivo* just through the plasma pharmacokinetics or lung drug exposure of tulathromycin. In our previous studies, good clinical efficacy of tulathromycin in the treatment of pneumonitis caused by *Pasteurella multocida* and in the treatment of pleuropneumonia caused by *Actinobacillus pleuropneumoniae* in pigs was observed (unpublished data). This clearly indicates an underestimation of *in vivo* antibiosis. As for the reason, it was interpreted that supplementation of the growth medium with serum, the pH of the culture media, and the presence of CO_2 might be the reasons for the underestimation *in vitro* antibiosises (Ednie *et al.*, 1998; Benchaoui *et al.*, 2004; Reese *et al.*, 2004; Godinho, 2008). The similar differences between *in vivo* and *in vitro* antibiosises were also noted in other macrolides such as erythromycin and azithromycin (Barry & Fuchs, 1991).

After oral administration, tulathromycin was detected quickly in the plasma, and it reached a maximum in about three to four hours and declined slowly. The T_{max} of tulathromycin after p.o. administration was 3.75 ± 0.710 h, which indicated that tulathromycin had been absorbed slowly. The value of geometric mean C_{max} was also lower than those obtained after i.m. administration in pigs (Benchaoui *et al.*, 2004) and cattle (Nowakowski *et al.*, 2004) and subcutaneous

Table 2. Inter- and intraday accuracy and precision for determination of tulathromycin and its metabolite in pig plasma (n = 15)

		Interday			Intraday		
Drug	Concentration ($\mu g \cdot L^{-1}$)	Found ($\mu g \cdot L^{-1}$)	SD	CV%	Found ($\mu g \cdot L^{-1}$)	SD	CV%
Tulathromycin	5.00	4.34	0.215	4.95	4.33	0.294	6.79
	125	119	4.83	4.06	118	11.5	9.75
	800	732	24.9	3.40	724	58.2	8.04
Tu-M	5.00	4.86	0.220	4.49	5.18	0.398	7.69
	125	122	3.57	2.93	128	7.35	5.74
	800	816	10.4	1.27	856	42.2	4.93

Tu-M, Marker residue of tulathromycin.

Table 3. Pharmacokinetic parameters after a single intravenous injection and an oral administration of tulathromycin of 2.5 mg/kg in pigs (mean ± SD)

Parameter	Intravenous injection	Oral administration
T_{max} (h)	0.0800	3.75 ± 0.710
C_{max} (μg·mL^{-1})	5.43 ± 2.71	0.200 ± 0.0500
AUC_{inf} (μg·h·mL^{-1})	4.67 ± 1.58	2.30 ± 0.540
AUC_{last} (μg·h·mL^{-1})	4.19 ± 1.43	1.93 ± 0.500
$AUMC_{last}$ (h·h·μg·mL^{-1})	195 ± 78.7	114 ± 29.6
$AUMC_{inf}$ (h·h·μg·mL^{-1})	351 ± 145	235 ± 39.0
MRT (h)	78.8 ± 16.7	104 ± 8.61
V_z (L/kg)	64.3 ± 21.2	NA
V_z_F (L/kg)	NA	130 ± 32.3
Cl (L/h/kg)	0.580 ± 0.170	NA
*Cl*_F (L/h/kg)	NA	1.14 ± 0.280
$t_{1/2\lambda z}$ (h)	76.5 ± 13.4	78.7 ± 6.75
λz (1/h)	0.00930 ± 0.00170	0.00890 ± 0.000800
Vss (L/kg)	44.6 ± 18.3	NA
F (%)	NA	51.1 ± 10.2

Data presented as mean ± standard deviation of 8 animals per treatment group. NA, not applicable; AUC_{last}, the area under the plasma concentration–time curve from time 0 to the last quantifiable time point (t_{last}); AUC_{inf}, the area under the plasma concentration–time curve from time 0 to infinity; $AUMC_{inf}$, the area under the first moment of the concentration–time curve extrapolated to infinity; $AUMC_{last}$, the area under the first-moment curve of the plasma drug concentration–time curve from zero to the last quantifiable time point (t_{last}); C_{max}, maximum plasma concentration; *Cl*, the total body clearance; T_{max}, time of occurrence of C_{max}; $t_{1/2\lambda z}$, the terminal half-life; λz, first-order rate constant associated with the terminal (log linear) elimination phase; *MRT*, mean residence time; F, bioavailability; V_z, the volume of distribution; *Vss*, the volume of distribution at steady state.

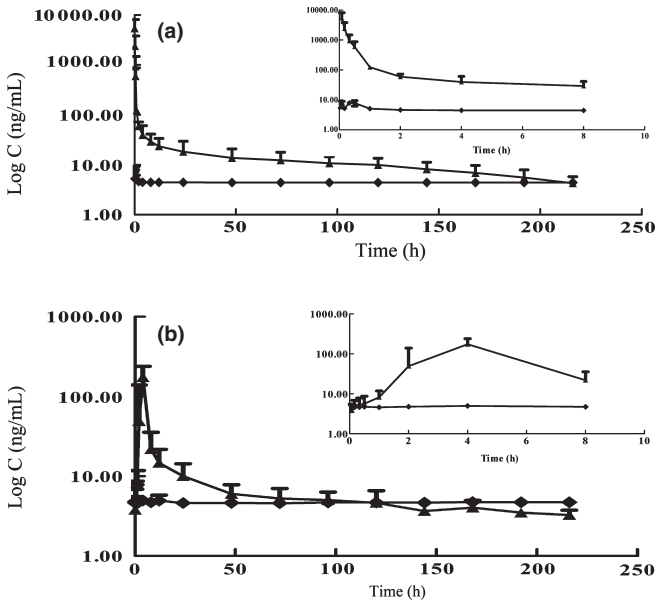

Fig. 4. Semi-log plot of concentration–time profiles of tulathromycin (▲) and metabolite of tulathromycin (◆) after an intravenous injection (a) and a single oral administration (b) to 8 pigs at a dose of 2.5 mg/kg, respectively.

administration in goats (Young *et al.*, 2011). Comparing the results, the $t_{1/2\lambda z}$ (78.7 ± 6.75 h) of tulathromycin after oral administration in pigs was similar to values reported after i.m. administration in pigs (75.6 ± 13.7 h) (Benchaoui *et al.*, 2004). It seemed that administration routes had little effects on the $t_{1/2\lambda z}$ in pigs. However, large differences in $t_{1/2\lambda z}$ were noted in animal species. The $t_{1/2\lambda z}$ after i.m. administration in cattle (100–110 h) was much longer than that obtained in pigs (Galer *et al.*, 2004).

In this study, low concentration of Tu-M was detected in plasma, which indicated that small amount of tulathromycin was hydrolyzed in swine. This phenomenon was in agreement with the previous research of tulathromycin using radiolabeled reference compounds (European Medicines Agency Veterinary Medicines and Inspections, EMEA, 2002). Because other metabolites of tulathromycin were not available, this analytical method did not detect other metabolites of tulathromycin in the plasma.

Usually, the drugs were administered after fasting to avoid the effect of food. Because most absorption occurs in the small intestine, gastric emptying is often the rate-limiting step. Food usually slows gastric emptying and decreases the rate of drug absorption. However, the effects of food on the pharmacokinetic parameters, such as bioavailability and peak concentration, were different in different drugs. Mean bioavailability of itraconazole (ITR) and its major metabolite hydroxyitraconazole (OH-ITR) were 43% and 38% higher, respectively, when ITR solution was taken as a single dose under fasted conditions (Barone *et al.*, 1998). Under the fed condition, following a single oral administration of clarithromycin and clarithromycin citrate salt in eight beagle dogs, delayed T_{max} and decreased C_{max} were noted, indicating that the consumption of this meal substantially reduced the drug's bioavailability (Zhang *et al.*, 2008). Lakritz *et al.* (2000) had found that the plasma concentrations, area under the plasma concentration–time curve, maximum plasma concentration, and estimated bioavailability of erythromycin A were higher in foals when food was withheld than when foals were fed (Lakritz *et al.*, 2000). Absorption was enhanced when erythromycin ethylsuccinate was given in milk (McCracken *et al.*, 1978). However, after a single oral dose of telithromycin 800 mg in healthy male subjects, the bioavailability, rate and extent of absorption of telithromycin were unaffected by food (Bhargava *et al.*, 2002). It was difficult to presume the pharmacokinetic changes after feeding based on the pharmacokinetic parameters when food was withheld. Therefore, pigs should be given tulathromycin solution before they were fed.

In conclusion, we firstly build a selective LC–MS/MS method for the quantification of tulathromycin and its metabolite in plasma in the pigs and demonstrate that the tulathromycin orally administrated to pigs is widely distributed and slowly eliminated. The absolute bioavailability of tulathromycin solution is 51.1 ± 10.2%. Furthermore, from this study, it is evident that small quantity of tulathromycin is quickly metabolized to its metabolite.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest. None of the authors of this manuscript has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the manuscript.

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