Received: 17 November 2020 | Revised: 16 January 2021 | Accepted: 4 February 2021

DOI: 10.1111/jvp.12958

JOURNAL OF Veterinary Pharmacology and Therapeutics WILEY

ORIGINAL ARTICLE

Pharmacokinetics and bioavailability of tildipirosin following intravenous and subcutaneous administration in horses

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Funding information
Jordan University of Science and Technology, Grant/Award Number: 298/2019

Abstract

This study was designed to investigate the safety and pharmacokinetic (PK) profile of tildipirosin in horses after intravenous (i.v.) and subcutaneous (s.c.) injection of a single dose at 4 mg/kg of body weight (b.w.). A total of 12 healthy mixed breed horses were used in the study. Horses were monitored for systemic and local adverse effects, and whole blood samples were collected for hematology and plasma biochemistry analysis at time (0) and at 6, 24, and 72 h after drug administration. For PK analysis, blood samples were collected at pre-determined times before and after tildipirosin administration. Plasma concentrations of tildipirosin were determined using ultra-high-performance liquid chromatography–ultraviolet detection method (UHPLC-UV). All horses tolerated the i.v. injection of tildipirosin without showing any systemic adverse effects. However, a non-painful, soft swelling appeared at the s.c. injection site in 5 horses (41.7%). On average, tildipirosin reached a maximum plasma concentration (C_{max}) of 1257 ng/ml (geometric mean) between 0.5 and 1.5 h after s.c. administration (T_{max}). The geometric mean values for total body clearance (Cl), the apparent volume of distribution based on the terminal phase (V_z), and the apparent volume of distribution at steady-state (V_{ss}) were 0.52 L/kg·h, 22 L/kg, and 10.0 L/kg, respectively. Data collected in this study suggests that tildipirosin can be used safely in horses with caution.

KEYWORDS
adverse effects, bacterial pneumonia, equine, macrolide, pharmacokinetics, tildipirosin

1 | INTRODUCTION

Tildipirosin, a semi-synthetic derivative of tylosin, belongs to the macrolide family of antibiotics that has been extracted from certain strains of *Streptomyces* bacteria found in soil (Elazab & Badawy, 2020). Tildipirosin is the most recent member of this group, which has been approved for the treatment of bacterial pneumonia in pigs and ruminants caused by sensitive strains of *Haemophilus parasuis*, *Mannheimia haemolytica*, *Pasteurella multocida*, and *Actinobacillus pleuropneumoniae* (Bartram et al., 2016; Confer et al., 2016; Rose et al., 2013; Teixeira et al., 2017; Torres et al., 2016; Zeng et al., 2018).

The newer generations of macrolide including tildipirosin have been characterized by excellent bioavailability, large tissue distribution, and long elimination half-life making them an excellent choice for single yet effective dosing regimens in animals (Abu-Basha et al., 2020; Elazab & Badawy, 2020; Galecio et al., 2020; Wang et al., 2017). Macrolide use in horses are viewed as of potential value in the treatment of respiratory tract diseases especially those caused by *Streptococcus equi* and *Rhodococcus equi* in foals

(Clark et al., 2008). The pharmacokinetic disposition of tilmicosin in horses following oral and s.c. administration at 10 mg/kg and 4 mg/kg b.w., respectively, has indicated poor absorption following oral administration but rapid and wide tissue distribution to the lungs, kidneys, liver, and muscle tissues following s.c. administration (Clark et al., 2008). Macrolide presents a major challenge in equine practice because of potential side effects including severe injection site reactions, fatal diarrhea, hyperthermia, and anhidrosis in foals (Pyörälä et al., 2014; Rakowska et al., 2020; Rutenberg et al., 2017; Stieler et al., 2016, 2017). Therefore, the search for newer and safer alternatives still exists. To our knowledge, there are no data that evaluated the safety and pharmacokinetics of tildipirosin in horses. Therefore, the objectives of this study were to evaluate the safety and pharmacokinetic profile of tildipirosin in horses following i.v. and s.c. administration. Data collected in this study are vital for planning of further research to investigate the clinical efficacy of tildipirosin against important respiratory pathogens of horses.

2 | MATERIALS AND METHODS

2.1 | Animals

All procedures performed in this study were reviewed and approved by the Jordan University of Science and Technology Animal Care and Use Committee which complies with local and international laws, regulations, and standards for use of animals in research (approval number 03–06–2019). A written and signed consent form was obtained from the farm owner before the start of the study.

This study was performed using 12 apparently healthy mixed breed horses (2 males and 10 non-pregnant females). The age of the horses ranged from 6 months to 15 years (0.5, 0.5, 1.5, 2, 5, 8, 10, 11, 12, 14, 14, and 15 years) and weighing between 150 to 560 kg. The horses belonged to a local horse-breeding farm. All horses were regularly vaccinated using a commercially available vaccine (Prevaccinol; MSD, Germany) and dewormed using fenbendazole (Panacur; Merck, USA) and ivermectin (Noromectin; Norbrook, Northern Irland) according to manufacturer's instructions. The last vaccination and deworming administration were performed 3 months prior to the commencement of the study. Horses were fed good quality hay and grain-based diet and offered freshwater ad libitum. Before enrollment in the study, horses were subjected to a complete physical examination. Horses selected for the study have had no recent history of respiratory or gastrointestinal diseases and were not administered any type of medications.

2.2 | Study design and drug administration

Horses were administered tildipirosin (Zuprevo 18%; Intervet Inc., Germany) i.v. and s.c. as a single dose at 4 mg/kg b.w. in a randomized, controlled crossover design with a 12-week washout period. Intravenous injections were administered in the right jugular vein and left neck area, respectively. The i.v. injections were performed as a bolus over 1 min using an 18 G 1.5 inch needles directly into the vein. Horses were monitored closely and immediately after drug administration for any adverse effects. The heart rate, respiration rate, and rectal temperature were determined once per day for 72 h after injections. The i.v. and s.c. injection sites were also inspected by palpation once per day for 1 week to detect any abnormal signs such as swelling, pain, heat, or discharge.

2.3 | Sample collection

Approximately, 8–10 ml of whole blood was collected via jugular venipuncture using syringes attached to a hypodermic needle and placed into heparinized blood collection glass tubes. Samples were collected at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 8, 12, and 24 h and at day 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, and 35 post-tildipirosin administration. Additional blood samples were taken at 5 min postdrug administration for i.v. route. Whole blood samples were also collected into EDTA containing blood collection tubes at 0, 6, 24, and 72 h and at day 7 after s.c. injection for hematology and plasma biochemistry analyses.

For pharmacokinetic study, whole blood samples were centrifuged immediately after collection at 3500 *g* for 10 min, and plasma samples were stored at −70°C until analysis was carried out.

2.4 | Hematology analysis

The following parameters were determined using automated hematology analyzer (Scil Vet ABC Hematology Analyzer, Scil Animal Care Company, USA): red blood cell count (RBC), packed cell volume (PCV), hemoglobin concentration (Hb), platelets count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and white blood cell count (WBC). The differential leukocyte count including the percentages of neutrophils, lymphocytes, monocytes, basophils, and eosinophils was determined manually using blood smears stained with Giemsa stain.

2.5 | Plasma biochemistry analysis

The following parameters were determined using commercially available kits and reagents (Randox Laboratories, UK) according to manufacturer's instructions: total protein, blood urea nitrogen (BUN), creatinine, aspartate transaminase (AST), alanine transferase (ALT), and alkaline phosphatase (ALP). In addition, the following plasma electrolytes were determined using automated chemistry analyzer (Awareness Technology, Inc., USA): calcium, potassium, magnesium, phosphorus, sodium, and chloride according to manufacturer's directions.

2.6 | Chromatographic conditions

The concentrations of tildipirosin in horse plasma were determined using ultra-high-performance liquid chromatography–ultraviolet detection method (UHPLC-UV) (Thermo Dionex-UltiMat 3000 controlled by chromeleon 7.2 software, Germany). The HPLC method and extraction procedures were modified from previously described method (Galecio et al., 2020).

All reagents, chemicals, solvents, drugs, and analytical standards used in this study were highly pure. Potassium hydrogen phosphate was bought from Sigma-Aldrich (Germany), diethyl ether and methanol (Tedia, USA), tildipirosin injectable grade (Catalogue No. BCP07404; BioChemPartner, China), formic acid (Merck, Germany), and tylosin phosphate was used as internal standard (Mobedco, Jordan). Ultrapure water was prepared in the laboratory using ultrapure water generator (Aqua Max, South Korea).

Chromatographic separation was performed using C18 column (HiQ Sil C18 HS 5 µm, 250 x 4.6; KYA Technologies Corporation, Japan) with gradient mobile phase. Mobile phase A consisted of 0.5% formic acid in water, and mobile phase B consisted of 0.5% formic acid in methanol. The mobile phase was filtered using a 0.45 µm membrane and degassed. A gradient solvent program with following conditions was applied: (i) 0–0.5 min (A-B 100:0 v/v); (ii) 0.5–9 min (A-B 40–60 v/v); (iii) 9–12 min (A-B 40–60 v/v); and (iv) 12–17 (A-B 100–0 v/v). The flow rate was performed at 1.3 ml/min, and the UV detector was set at a wavelength of 289 nm. The volume of injection was 80 µl.

2.7 | Sample preparation

The frozen plasma samples were thawed at room temperature. Then, 700 µl of plasma was added to 100 µl of tylosin (100 µg/ml) and 200 µl di-potassium hydrogen phosphate (0.1 M) in a clean glass tube. Then, 3 ml of diethyl ether was added to the mixture, vortexed for 1 min, and centrifuged for 5 min at ~1000 *g*. The organic layer was decanted and removed into clean glass tube. The samples were evaporated to dryness using centrifugal rotary evaporator at 40°C, and then reconstituted with 200 µl of water: methanol (50:50) and placed in flat bottom glass insert 250 µl. Then 80 µl were injected in the UHPLC-UV.

2.8 | Calibration curve

Daily fresh calibration curves were prepared as described in sample preparation. The standard tildipirosin stock solution was prepared to obtain a concentration of 100 µg/ml. This solution was used to prepare standards of 10, 50, 100, 500, 1000, 5000, 10,000 and 20,000 ng/ml in ultrapure water or drug-free horse plasma. The calibration curves were determined by plotting the peak area as a function of the respective tildipirosin concentrations, and the linear regression was calculated.

2.9 | Methods validation

The validation of tildipirosin analytical methods was performed by assessing linearity, accuracy, precision, recovery, and sensitivity. Replicates were prepared for tildipirosin at concentrations of 10, 80, 800, and 8000 ng/ml. Linear equations of calibration curve of tildipirosin were calculated from standard level point areas versus level concentrations. The accuracy was calculated using the following equation (Bennett & Briggs, 2018): accuracy (%) = 100 × (measured value −theoretical value)/theoretical value. The sensitivity was determined by the lowest concentration of tildipirosin that can be measured with acceptable accuracy and precision. The calculated limit of detection (LOD) was determined based on a signal-to-noise ratio of 3 or 2:1; whereas, the limit of quantification (LOQ) of tildipirosin was determined based on a signal-to-noise ratio of 10:1.

The intra- and inter-day precision assays were performed by measuring the coefficient of variation (CV %) of tildipirosin at lower limit of quantification (LLOQ), low, medium, and high concentrations. Six replicates of tildipirosin were used for intra- and inter-day precision assays at 10, 80, 800, and 8000 ng/ml for LLOQ, low, medium, and high concentrations, respectively.

2.10 | Pharmacokinetic analysis

The pharmacokinetic parameters of tildipirosin were calculated by non-compartmental analysis using Phoenix 8.3 (Certara, USA) according to the previously published methods (Ganti et al., 2013). The area under the plasma concentration–time curve and the area under the first moment curve from zero to time of last measurable concentration (AUC_{last}, $AUMC_{last}$; h·mg/L, h·h·mg/L, respectively) were calculated using the linear log trapezoidal method for the i.v. data and the linear up/log down method for the s.c. data; the terminal slope of the time–concentration curve was determined by linear regression (λ_z; 1/h), and the terminal half-life ($T_{½\lambda z}$; h) was calculated using the equation $T_{1/2\lambda_z} = \frac{0.693}{\lambda_z}$. The AUC was extrapolated to infinity ($AUC_{0\text{-}\infty}$) by adding the term $\frac{C_{last}}{\lambda_Z}$ to $AUC_{0\text{-last,}}$ where C_{last} is the last observed concentration. The AUMC was extrapolated to infinity ($AUMC_{0\text{-}\infty}$) by adding the term $\frac{t_{last} \times C_{last}}{\lambda_Z} \times \frac{C_{last}}{\lambda_Z^2}$. The following parameters were determined based on observed data (Won et al., 2018): maximum concentration (C_{max}; ng/ml), and time to C_{max} (T_{max}; h). Total body clearance (Cl; L/h/kg) and volume of distribution based on the terminal phase (V_z; L/kg) were calculated using the equations $Cl = \frac{Dose}{AUC_{0-\infty}}$ and $V_Z = \frac{Dose}{\lambda_Z \times AUC_{0-\infty}}$. The volume of distribution at steady-state (Vss; L/kg) was calculated using the equation $V_{SS} = \frac{MRT_{0-\infty}}{C_{last}}$.

2.11 | Statistical analysis

Data related to the hematology, plasma biochemistry analyses, and tildipirosin plasma concentration–time are presented as

means ± deviation (mean ± SD). Significant differences in values of blood and plasma biochemistry parameters before and after administration of the drug were determined using repeated-measures analysis of variance (ANOVA). All statistical analyses were performed using SPSS version 23 software (IBM SPSS Statistics, USA). Values were considered significant at $p \leq 0.05$.

The geometric mean and standard deviation of the log-transformed values were calculated as measures of central tendency and spread of the pharmacokinetic parameters since these are typically log-normally distributed (Julious & Debarnot, 2000).

3 | RESULTS

Horses tolerated a single i.v. and s.c. dose of tildipirosin at 4 mg/kg b.w. without showing any adverse clinical signs except for mild subcutaneous injection site reaction. The heart rate, respiration rate, and rectal temperature remained within normal limits following injection. A variable size (10–15 cm in diameter) swelling at the s.c. injection site appeared in 5 out of 12 horses (41.7%) (Figure 1). The swelling developed slowly and progressively within 30–60 min following injection of the drug. The swelling appeared non-septic (no abnormal discharge), soft and pitting, and not painful. The swelling required no special treatment and disappeared within approximately 48–72 h postdrug administration. Long-term monitoring of the s.c. injection site revealed no long-lasting adverse effects such as abscess development.

The hematology and plasma biochemistry analyses revealed no statistically significant changes in any of the parameters except for the percentage of eosinophils (Tables 1 and 2). Eosinophil percentages were significantly ($p \leq 0.05$) increased 6 h after injection and remained significantly elevated 72 h after injection. On day 7, the percentage of eosinophils returned to baseline levels.

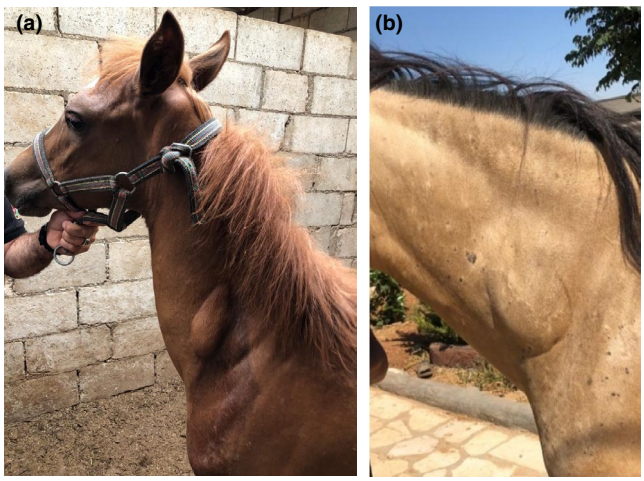

FIGURE 1 Non-painful, soft tissue swelling at the s.c. injection site post-tildipirosin administration (4 mg/kg b.w.) in horses. The swelling appeared within 30 min after injection in 5 out of 12 (41.7%) horses. The swelling disappeared without special treatment within 48–72 h after injection [Colour figure can be viewed at wileyonlinelibrary.com]

Tildipirosin calibration curve was linear through the concentrations 10–20,000 ng/ml with a correlation coefficient of ≥0.999. The LOD and LOQ of tildipirosin were 3 and 10 ng/ml, respectively. The percentage analytical recovery of tildipirosin in plasma ranged from 79% to 82% for low, medium, and high concentrations, whereas, 86% for internal standard. The intra-day and inter-day assay coefficients of variations were <20% for LLOQ and <10% for low, medium, and high concentrations. The accuracy was 81%, 82.8%, 95.3%, and 97.5% for LLOQ, low, medium, and high concentration, respectively. The method was specific since there were no interfering peaks present in chromatograms corresponding to the retention time at 5.9 and 11.4 min for tildipirosin and internal standard, respectively.

The plasma concentrations, plasma concentration–time profile, and pharmacokinetic parameters of tildipirosin after a single i.v. and s.c. administration at 4 mg/kg b.w. in horses are presented in Tables 3 and 4, Figure 2. Tildipirosin was detected up to 72 h in all tested horses after i.v. administration and up to 28 days after s.c. administration in 9 out of 12 horses. Tildipirosin reached an average (geometric mean) maximum plasma concentration (C_{max}) of 1257 ng/ml between 0.5 and 1.5 h following administration (T_{max}). The terminal half-life was longer following s.c. administration (geometric mean of 170 h) than following i.v. administration (geometric mean of 30 h). The terminal half-life was also more variable following s.c. administration (standard deviation of the log-transformed values 0.55) compared to i.v. administration (standard deviation of the log-transformed values 0.18), as a result, the bioavailability of tildipirosin following s.c. administration was overestimated (>400%).

After i.v. administration of tildipirosin, the geometric mean values of the total body clearance (Cl), the apparent volume of distribution based on the terminal phase (V_z), and the apparent volume of distribution at steady-state (V_{ss}) were 0.52 L/kg·h, 22 L/kg, and 10.0 L/kg, respectively.

4 | DISCUSSION

In equine practice, there has been an increased interest over the years in the use of different macrolide for the treatment and control of respiratory diseases especially in foals affected with *Rhodococcus equi* (Cohen, 2014; Rakowska et al., 2020; Reuss & Cohen, 2015; Rutenberg et al., 2017). Erythromycin in combination with rifampicin has been the standard treatment of this important disease for over 30 years (Stieler et al., 2016). Newer macrolide such as clarithromycin and azithromycin has offered more preferred choices because of better bioavailability, longer activity, and wider tissue distribution (Rakowska et al., 2020; Rutenberg et al., 2017; Stieler et al., 2016). In this study, tildipirosin was administered to 12 apparently healthy horses ranging in age from 6 months to 15 years to determine its safety and pharmacokinetics for the first time. Horses appeared to tolerate the i.v. and s.c. administration of a single dose of tildipirosin at 4 mg/kg b.w. without showing any systemic adverse clinical signs related to the gastrointestinal tract, body temperature, heart rate, or respiration

TABLE 1 Mean ± SD of hematology parameters in healthy horses administered a single dose of tildipirosin s.c. at 4 mg/kg b.w. (n = 12)

Parameters	Reference ranges[a]	Time points				
		Before	6 h	24 h	72 h	Day 7
WBC (×10^3 cells/μl)	4.9–10.3	7.8 ± 2.3	8.6 ± 3	7.8 ± 2.8	8.9 ± 3.5	7.2 ± 1.6
RBC (×10^6 cells/μl)	6.2–10.2	8.2 ± 1.9	8.6 ± 1.6	8.9 ± 1.7	8.6 ± 1.7	8.4 ± 1.9
Hb (g/dl)	11.4–17.3	10.8 ± 1.1	12.8 ± 1.5	12 ± 1.1	12 ± 1.5	11.5 ± 0.8
PCV%	31–50	32 ± 3.7	38 ± 4.3	37 ± 3	35 ± 4.6	34 ± 2.9
Platelet (×10^3 cells/μl)	72–183	143 ± 21	171 ± 70	180 ± 34	180 ± 28	164 ± 39
Lymphocytes (%)	19.8–58.9	51 ± 12	42 ± 18	45 ± 13	50 ± 10	40 ± 8.7
Neutrophils (%)	28.0–82.8	46 ± 9	50 ± 16	51 ± 14	50 ± 8	53 ± 10
Monocytes (%)	1.4–10.5	2.4 ± 0.5	4 ± 1.3	3.4 ± 1.6	2.4 ± 1.2	4.2 ± 1.8
Eosinophils (%)	0–8.7	0.3 ± 0.06	3.3 ± 2.0*	3.7 ± 1.2*	2.4 ± 1.1*	0.3 ± 0.03

Abbreviations: Hb, hemoglobin concentration; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; RBC, red blood cell count; WBC, white blood cell count.

[a]Harvey (1990).

*$p \leq 0.05$.

TABLE 2 Mean ± SD of plasma biochemistry parameters in healthy horses administered a single dose of tildipirosin s.c. at 4 mg/kg b.w. (n = 12)

Parameters	Reference ranges[a]	Time points				
		Before	6 h	24 h	72 h	Day 7
AST (U/L)	205–555	122 ± 8	137 ± 32	144 ± 32	115 ± 30	141 ± 52
ALT (U/L)	0.3–7.0	6.8 ± 3.5	3.5 ± 2.8	3.3 ± 2.3	3.7 ± 2.3	6.4 ± 2.6
ALP (U/L)	109–315	256 ± 80	290 ± 100	280 ± 79	240 ± 80	310 ± 120
Creatinine (mg/dl)	0.6–1.8	1.2 ± 0.6	1.3 ± 0.3	1.3 ± 0.3	1.4 ± 0.3	1.3 ± 0.4
BUN (mg/dl)	8–27	22 ± 2	20 ± 4.6	18 ± 4.3	20 ± 5.3	24 ± 7
Total protein (g/dl)	4.6–6.9	75 ± 4.4	74 ± 3.3	80 ± 4.6	77 ± 3.4	75 ± 3.6
Sodium (mM)	132–141	131 ± 1.6	132 ± 0.1	135 ± 1.6	130 ± 3.4	134 ± 3
Potassium (mM)	132–141	3.5 ± 0.4	3.8 ± 0.3	3.2 ± 0.6	3.6 ± 0.5	3.5 ± 0.4
Chloride (mM)	94–102	103 ± 2.7	103 ± 1.6	106 ± 2	104 ± 1.9	103 ± 3.4
Calcium (mg/dl)	10.7–13.4	12.2 ± 1.4	11.6 ± 1.4	11.3 ± 1.1	12.5 ± 0.9	12.2 ± 1.2
Magnesium (mg/dl)	1.6–2.5	1.9 ± 0.2	2 ± 0.1	2 ± 0.1	2 ± 0.4	1.9 ± 0.3
Phosphorus (Mg/dl)	1.9–5.4	4.2 ± 1.4	3.4 ± 1.5	4.2 ± 2.3	4.3 ± 1.5	4.3 ± 1.7

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen.

[a]Bauer (1990).

rate. Furthermore, serial complete blood cell count and plasma biochemistry analyses revealed no significant abnormalities except increased percentage of eosinophils during the first 72 h following drug administration. Eosinophilia detected in this study is difficult to explain. Interestingly, in human literature, there is a controversy on the effect of macrolide on the general inflammatory response and peripheral eosinophil count. While earlier in vivo studies have shown that macrolide may decrease eosinophilic inflammatory reaction and eosinophilic count in human patients with asthma and rhinitis (Wallwork & Williamb, 2004), other more recent research has reported a significant increase in blood eosinophil count in patients with chronic, non-septic pulmonary inflammation treated with macrolide (Asciak et al., 2017). Furthermore, earlier in vitro studies have indicated possible anti-inflammatory activities of macrolide in addition to their antibacterial effects (Wallwork & Williamb, 2004).

In this study, 5 horses out of 12 (41.7%) developed a variable size (10–15 cm in diameter) local swelling immediately after s.c. injection that persisted for 48–72 h. The swelling disappeared in all affected horses without the need for any special treatment, and therefore, further investigations by ultrasonographic examination or sample collection for potential cytology or bacterial culture were not carried out. Clinically, the swellings were not painful and appeared pitting indicating an edematous nature of the problem. Inflammation due to s.c. injection of the drug was expected as the cause of this swelling which may also explain the eosinophilia reported in this study. However, the effect of tildipirosin on the general inflammatory response, particularly on eosinophil functions,

TABLE 3 Tildipirosin plasma concentrations (ng/ml) in horses after a single intravenous (i.v.) and subcutaneous (s.c.) administration at 4 mg/kg b.w. Values are means ± SD (n = 12)

	Route of administration and dosage	
Time (h)	i.v.	s.c.
0.833	9332.12 ± 1587.96	–
0.25	3635.18 ± 1072.17	810.55 ± 136.45
0.5	1796.12 ± 381.68	1153.11 ± 143.24
1	992.56 ± 137.16	1306.26 ± 132.71
1.5	681.39 ± 160.28	1147.27 ± 122.07
2	364.84 ± 65.11	939.49 ± 90.44
3	284.85 ± 50.80	716.70 ± 63.33
4	236.94 ± 31.05	555.0 4 ± 45.72
6	217.39 ± 32.98	502.92 ± 42.54
8	137.99 ± 24.86	400.89 ± 35.61
12	70.75 ± 13.68	313.81 ± 38.59
24	34.84 ± 4.87	156.10 ± 12.24
48 (day 2)	18.69 ± 2.06	90.75 ± 8.85
72 (day 3)	15.39 ± 3.35	72.72 ± 8.04
96 (day 4)	–	66.02 ± 8.50
120 (day 5)	–	58.35 ± 9.53
144 (day 6)	–	52.64 ± 7.59
168 (day 7)	–	48.25 ± 7.51
192 (day 8)	–	45.58 ± 8.04
216 (day 9)	–	42.37 ± 8.32
240 (day 10)	–	41.60 ± 8.65
288 (day 12)	–	20.43 ± 0.94
336 (day 14)	–	20.01 ± 0.77
384 (day 16)	–	17.91 ± 0.80
432 (day 18)	–	16.70 ± 0.89
480 (day 20)	–	16.76 ± 1.10
528 (day 22)	–	16.42 ± 1.00
576 (day 24)	–	16.48 ± 0.78
624 (day 26)	–	15.18 ± 0.70
672 (day 28)	–	15.25 ± 0.54
840 (day 35)	–	–

TABLE 4 Geometric mean (SD of the natural log) of various pharmacokinetic parameters of tildipirosin after a single intravenous (i.v.) and subcutaneous (s.c.) administration at 4 mg/kg b.w. in horses (n = 12)

		Route of administration	
Parameter	Units	i.v.	s.c.
C_{max} (range)	ng/ml	–	634–1954
T_{max} (range)	h	–	0.5–1.5
$AUC_{0-\infty}$	h·ng/ml	7730 (0.4)	31014 (0.5)
AUC% (extrapolated)	%	6 (0.7)	15 (0.6)
λ_z	1/h	0.02 (0.4)	0.004 (1.3)
$T_{½\lambda z}$	h	29 (0.4)	170 (1.2)
$MRT_{0-\infty}$	h	19 (0.7)	193 (1.2)
V_z	L/kg	22 (0.6)	–
Cl	L/h·kg	0.52 (0.4)	–
V_{ss}	L/kg	10.0 (0.9)	–

Abbreviations: AUC% (extrapolated), percentage of AUC due to extrapolation from T_{last} to infinity; $AUC_{0-\infty}$, area under the plasma concentration–time curve from zero to time infinity; Cl, clearance; C_{max}, maximum concentration; $MRT_{0-\infty}$, mean residence time from the time zero to infinity; $T_{½\lambda z}$, terminal half-life; T_{max}, time to C_{max}; Vss, volume of distribution at steady-state; Vz, volume of distribution; λ_z, terminal rate constant.

in horses remains to be investigated. Similar but more severe injection site reactions have been reported following s.c. administration of tilmicosin in horses (Clark et al., 2008). Therefore, careful monitoring of horses after tildipirosin s.c. injection is warranted in the field.

The pharmacokinetic profiles of tildipirosin in horses have not been determined previously. In this study, the disposition of tildipirosin following s.c. injection indicated variable and prolonged absorption with C_{max} ranging from 634 to 1954 ng/ml between 0.5 and 1.5 h after administration. The terminal half-life was considerably longer following s.c. administration than following i.v. administration (geometric mean of 170 h compared to 30 h, respectively). Furthermore, the inter-individual variability of the terminal slope was very high following s.c. administration, ranging from 4.7 to 631 h. This is most likely due to the flip-flop kinetics phenomena in which the rate of absorption following extravascular (s.c.) administration is slower than the rate of elimination, resulting in the terminal slope of the plasma concentration–time curve being driven by absorption and not clearance and volume of distribution (Yáñez et al., 2011). As a result, the bioavailability data could be overestimated (>400% in this study) which, clinically might lead to the emergence of drug-resistant bacteria. It is well-known that the ratio of the area under the concentration–time curve from 0 to 24 h to the MIC (AUC0–24: MIC ratio) can be used as an important predictor of bacterial resistance for antimicrobials that are time-dependent. When the AUC0–24: MIC ratio is <100, the chances of bacterial resistance increase significantly during the treatment. Therefore, one should be cautious when interpreting high bioavailability when it is overestimated.

The terminal half-life of tildipirosin after s.c. administration in horses determined in this study (7 days) was similar to those determined in cattle (9 days), and sheep (6 days), and longer than in swine (4 days) (Abu-Basha et al., 2020; Giguère et al., 2011; Menge et al., 2012; Pyörälä et al., 2014). Moreover, the half-life of tildipirosin in horses is longer than other newer generations of macrolide such as tulathromycin (4 days) and gamithromycin (3 days) reported in cattle (Pyörälä et al., 2014). Drug formulation and interspecies variations in drug absorption and metabolism account for such differences in terminal half-life between different drugs and different animal species.

Similar to tildipirosin, the pharmacokinetic studies of azithromycin, gamithromycin, or tulathromycin in various animal species have

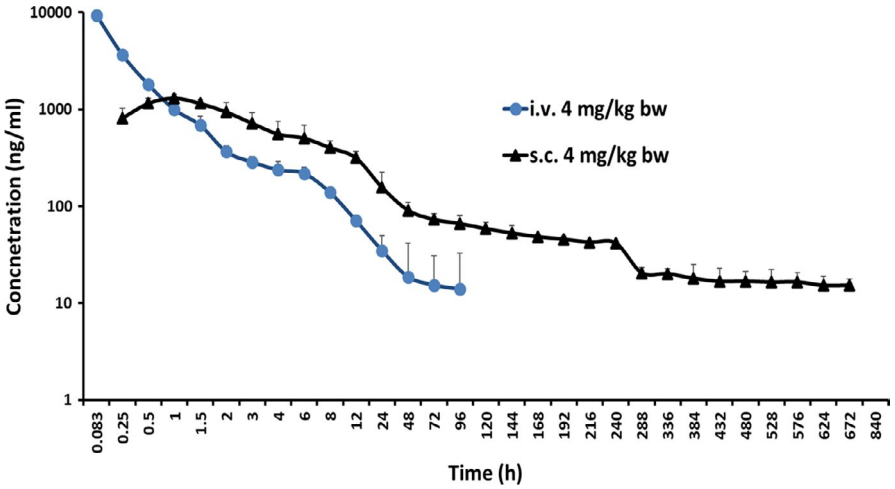

FIGURE 2 Semilogarithmic plot showing the plasma concentration–time profile of tildipirosin after a single intravenous (i.v.) and subcutaneous (s.c.) administration at 4 mg/kg b.w. in horses. Values are means ± SD (n = 12) [Colour figure can be viewed at wileyonlinelibrary.com]

indicated wide tissue distribution with very high tissue concentrations, usually above the MIC of most susceptible bacterial species (Bladek et al., 2015; Galecio et al., 2020; Yang et al., 2019). For example, in swine and cattle, tildipirosin concentrations following s.c. administration have been reported 31 times higher in the respiratory tissues than in plasma at 120 h after a single drug administration (Galecio et al., 2020). In this study, the apparent volume of distribution of tildipirosin in horses (22 L/kg) is higher than that reported in cattle, sheep, goats, pigs, rabbits, and dogs (Menge et al., 2012; Lei et al., 2017; Wang et al., 2017; Galecio et al., 2020; Xiong et al., 2020). High bioavailability and tissue distribution have been viewed as an essential feature that explain an expected high efficacy of macrolide against intracellular or tissue invading bacteria (Galecio et al., 2020). Based on the findings of bioavailability data of tildipirosin, high tissue concentrations following a single s.c. dose could be presumed to occur in horses. However, further research is still warranted to prove such an assumption.

In conclusion, results of this study indicate that i.v. and s.c. administration of tildipirosin at 4 mg/kg to horses aging between 6 months and 15 years resulted in no systemic adverse effects. However, s.c. injection of the drug caused local tissue reaction and resulted in prolonged and variable absorption. This mild tissue reaction warrants close monitoring upon clinical use of tildipirosin in horses in the field.

ACKNOWLEDGMENTS

This work was financially supported by the Deanship of Research at Jordan University of Science and Technology (grant #298/2019). The software license for Phoenix® was provided through the Certara Centers of Excellence program (USA).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

EAB experimental design, laboratory analysis, data interpretation, and corresponding author. ZBI sample collection, scientific, and language editing of the manuscript. MA sample collection and clinical monitoring of horses following treatment. EH laboratory analysis. RG statistical analysis, pharmacokinetic parameters analysis, and interpretation.

ETHICAL APPROVAL

This study was approved by the Institutional Animal Care and Use Committee of Jordan University of Science and Technology (JUST-ACUC) which complies with local and international laws, regulations, and standards for use of animals in research (approval number 03–06–2019).

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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How to cite this article: Abu-Basha EA, Bani Ismail Z, Ababneh MM, Hamzeh E, Gehring R. Pharmacokinetics and bioavailability of tildipirosin following intravenous and subcutaneous administration in horses. *J Vet Pharmacol Therap*. 2021;44:544–551. https://doi.org/10.1111/jvp.12958