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# Pharmacokinetics and bioavailability of tildipirosin in goats using HPLC

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## Abstract

The goal of this study was to assess the pharmacokinetic properties of tildipirosin in female goats (n=15) following single intravenous (IV) injection at 4mg/kg and subcutaneous (SC) administration at 2 and 4 mg/kg. There were no adverse effects noted after IV and SC administration. Plasma concentrations of tildipirosin were measured using high-performance liquid chromatography (HPLC). The pharmacokinetic parameters of tildipirosin were computed by the non-compartmental model with WinNonlin 4.1 software. After single SC injection of 2 and 4 mg/kg, the maximum plasma concentrations were  $571.6 \pm 39.22$  and  $720 \pm 52.99$  ng/ml achieved at 30 min, respectively. Moreover, the mean values for the elimination half-life ( $T_{1/2\lambda z}$ ) were  $75.34 \pm 2.71$  and  $94.76 \pm 8.13$  hr, and the  $AUC_{0-last}$  values were  $11.09 \pm 1.64$  and  $19.59 \pm 4.18$  respectively. The absolute bioavailability of tildipirosin after 4mg/kg SC injection was 96.64%. Tildipirosin was widely distributed following 4 mg/kg IV injection with volume of distribution  $24.69 \pm 4.10$  L/kg and the plasma clearance was  $0.216 \pm 0.029$  L/hr/kg. Tildipirosin showed favorable pharmacokinetic features in goats with rapid absorption, high bioavailability, wide distribution and long persistence in the body. These findings provide a guidance for using tildipirosin in goats.

Key Words: Goat, HPLC, Pharmacokinetics, Tildipirosin.

## Introduction

Macrolides, an antibiotic family, are frequently utilized in veterinary field<sup>4,8,20</sup>. It includes several structurally related compounds, most of them were extracted from different strains of *Streptomyces* soil-born bacteria<sup>18</sup>. Tildipirosin, a recent 16-membered-ring macrolide, is a semisynthetic derivative of tylosin introduced into veterinary market for curing and controlling respiratory tract infections caused by

several pathogens such as *Haemophilus parasuis*, *Mannheimia haemolytica*, *Pasteurella multocida*, and *Actinobacillus pleuropneumoniae*<sup>16,19</sup>. It produces its bacteriostatic effect by suppressing the bacterial protein synthesis through binding to the 23S ribosomal ribonucleic acid (rRNA) of the 50S ribosomal subunit of bacterial cells<sup>17</sup>. Tildipirosin has been licensed as a therapy for respiratory diseases in cattle and swine<sup>6</sup>.

Tildipirosin is characterized by its rapid absorption and extensive distribution into body

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**Table 1. Validation parameters of the HPLC method**

Parameter	Unit	
Recovery	%	85-98
Intra-day RSD	%	2.514
Inter-day RSD	%	2.87

fluids and tissues<sup>6,22</sup>). To our knowledge, there are no data assessed the pharmacokinetics of tildipirosin in goats, unlike other species as cattle<sup>13</sup>, pig<sup>11,16</sup>, and dogs<sup>21</sup>).

Understanding the pharmacokinetic behavior of tildipirosin in goats may allow for planning of future studies to explore the potential use of tildipirosin in goats as there is an urgent need for new potent antibiotic to overcome resistant pathogens. Therefore, the purpose of this study was to determine the pharmacokinetic profile of tildipirosin in goats following intravenous (IV) and subcutaneous (SC) administration using high performance liquid chromatography (HPLC).

## Materials and Methods

### *Drugs and chemicals:*

Tildipirosin (Zuprevo<sup>®</sup>, 180 mg/ml, Intervet International GmbH, a member of the MSD Animal Health, Germany) was utilized in this study. The tildipirosin reference standard was provided from Intervet International Company, Cairo, Egypt. HPLC analytical grade acetonitrile, formic acid, dipotassium hydrogen phosphate, and diethyl ether were procured from Merck, Darmstadt, Germany. Water utilized for HPLC investigation was purified using a Milli-Q system (Waters Corp., Milford, MA, USA).

### *Animals:*

The current study employed fifteen, clinically healthy females, non-pregnant, non-lactating, Baladi goats (aged  $1.5 \pm 0.3$  year and  $22 \pm 2$  kg body weight). Goats were kept in suitable stable and had not been treated with any medication for

the 2 months before the onset of the study. Their diet during the experiment composed of barely grain and green feed with water ad libitum. The Ethics Committee of the Faculty of Veterinary Medicine (Mansoura University, Mansoura, Egypt) approved the animal study.

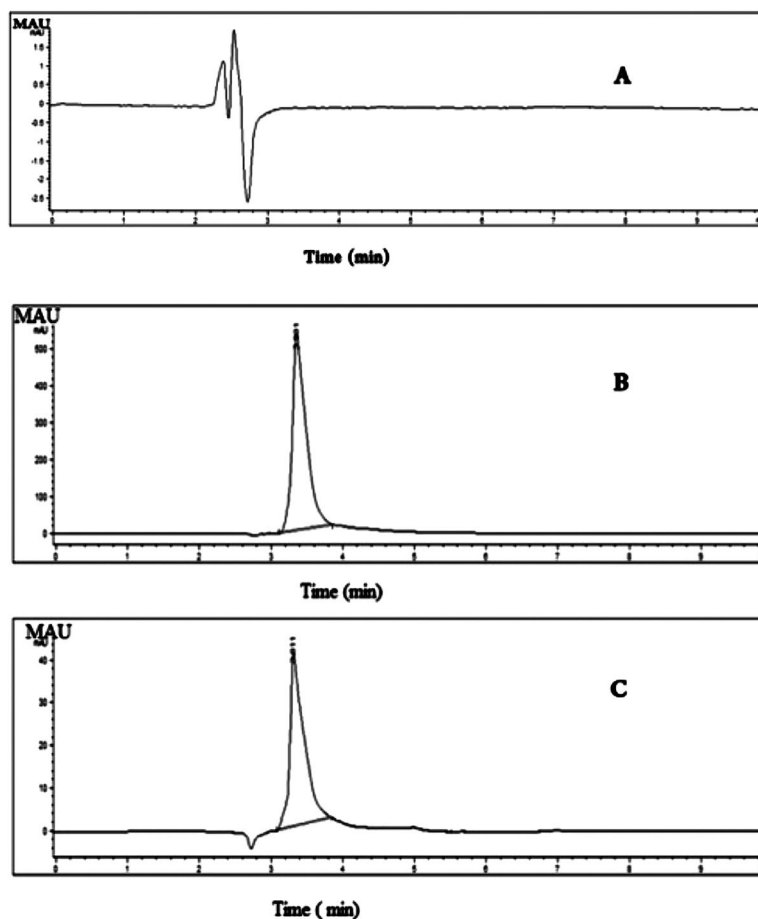
### *Experimental design:*

The goats were equally divided into three groups; each consisted of 5 goats. A parallel study design was followed. In the first group, goats received a single dose of tildipirosin at 4 mg/kg by IV route. In the second group, goats were injected subcutaneously with a single dose of tildipirosin at 4 mg/kg. While, goats in the third group were administered a single dose of tildipirosin subcutaneously at 2 mg/kg. Tildipirosin was injected into the right jugular vein and in the right neck for SC administration. Serial blood samples of 2 ml each were drawn from the contralateral vein of each goat at time 0 (before tildipirosin administration), 15, 30 min, 1, 2, 4, 8, 10, 18 hr, 1, 3, 4, 6, 8, 10, 14, 16, and 18 days post-tildipirosin administration. Additional samples were collected from goats in the first group at 5 min after IV injection. Blood samples were collected into heparinized tubes and centrifuged at 3000 g for 10 min. The plasma samples were stored at -80°C until analysis. *After tildipirosin injection, the goats were examined for any noticeable adverse effects at the site of injection (irritation and inflammation) and for any alteration in the physical parameters including temperature, pulse, respiration, appetite and fecal consistency.*

### *Analysis of tildipirosin in plasma samples:*

#### *Preparation of plasma samples*

Tildipirosin stock solution was prepared at concentration of 1 mg/mL of tildipirosin base. Tildipirosin calibration standards were prepared at concentrations of 0, 1, 13.5, 27, 67.5, 135, 270, 675, 1350, 1800, 2700 and 5400 ng/mL utilizing blank goat plasma as the solvent. Preparation of plasma samples was carried out



**Fig. 1** Revealing liquid chromatogram of 50  $\mu$ l injection of (A) blank plasma sample (without tildipirosin). (B) tildipirosin standard 5400 ng/ml. (C) tildipirosin extract from goat plasma at 4 hours after a single Sc administration at a dose of 4 mg/kg.

according to previously published method<sup>11</sup>. Briefly, 500  $\mu$ l aliquot of standard in blank plasma or plasma sample was added to 200  $\mu$ L of dipotassium hydrogen phosphate (0.1 mol/L), then the extraction was performed using diethyl ether. After centrifugation, the supernatant was evaporated to dryness and re-suspended with 0.1 ml acetonitrile. Then, 50  $\mu$ l was injected into the HPLC.

#### *Chromatographic conditions*

The plasma concentrations of tildipirosin were determined following a previously reported HPLC method<sup>11</sup> *with some modifications*. The HPLC Agilent Series 1200 quaternary gradient pump, Series 1200 autosampler, Series 1200 UV VIS detector set at 289 nm, and HPLC 2D chemstation software (Hewlett-Packard, Les Ulis,

France) were utilized. A Phenomenex C18 (5  $\mu$ m, 250 mm x 4.6 mm) was used for chromatographic separation. The mobile phase comprised 0.15% formic acid in acetonitrile with isocratic method. The flow rate was 1.2 ml/min. The method was revalidated following the EMA instructions<sup>11</sup> utilizing goat plasma (Table 1). Linearity of the method was observed ( $R^2 > 0.99$ ) in the range of 1-5400ng/ml. The retention time was 3.3 min. The lower limit of detection of tildipirosin was 1ng/ml.

#### *Pharmacokinetic analysis:*

The pharmacokinetic parameters of tildipirosin were computed by the non-compartmental model<sup>11,13,21</sup> with WinNonlin 4.1 software (Pharsight, CA, USA). After Sc injection, the maximum plasma concentration

**Table 2. Pharmacokinetic parameters of tildipirosin in goats after single SC administration of 2 and 4 mg/kg and IV injection at 4 mg/kg BW.**

Parameters	SC 2 mg/kg	SC 4mg/kg	IV 4 mg/kg
$C_{max}$ (ng/ml)	571.6 ± 39.22	720 ± 52.99	—
$T_{max}$ (hr)	0.5 ± 0.00	0.5± 0.00	—
$\lambda z$ (1/hr)	0.009 ± 0.0004	0.007 ± 0.0006	0.009± 0.0005
$T_{1/2} \lambda z$ (hr)	75.34 ± 2.71	94.76 ± 8.13	77.89 ± 4.29
$AUC_{0-last}$ ( $\mu g \cdot hr/ml$ )	11.09 ± 1.64	19.59 ± 4.18	20.27 ± 3.38
$AUC_{0-\infty}$ ( $\mu g \cdot hr/ml$ )	11.36 ± 1.67	20.41 ± 4.38	20.71 ± 3.39
$V_z$ (L/kg)	—	—	24.69 ± 4.10
Cl (L/hr/kg)	—	—	0.216 ± 0.029
MRT (hr)	72.88 ± 5.38	86.14 ± 7.29	56.57 ± 2.88
AUMCINF ( $hr \cdot hr \cdot \mu g/ml$ )	964.95 ± 176.74	2255.28 ± 563.46	1400.02 ± 262.07
F (%)	—	96.64	—

$C_{max}$ : Peak plasma;  $T_{max}$ : Time to peak concentration;  $\lambda z$ : , the first order rate constant;  $T_{1/2} \lambda z$ : elimination half-life;  $AUC_{0-last}$ : area under the plasma concentration vs. time curve from 0 to last time;  $AUC_{0-\infty}$ : Area under the plasma concentration-time curve from 0 to infinite;;  $V_z$ : apparent volume of distribution in terminal phase; Cl: total body clearance; MRT: Mean residence time; AUMC: Area under the first moment curve; F mean systemic bioavailability. Data are expressed as mean ± SEM (n = 5).

( $C_{max}$ ), and the time required to reach  $C_{max}$  ( $T_{max}$ ), were defined by visual examination of the plasma concentration-time profile. The area under the plasma concentration- time curve ( $AUC_{0-\infty}$ ) was assessed using the trapezoidal method. The elimination half-life ( $T_{1/2} \lambda z$ ) was determined using the equation:  $T_{1/2} \lambda z = 0.693/\lambda z$ , where  $\lambda z$ , the first order rate constant, was measured from the terminal slope of the plasma concentration-time curve. The clearance (Cl) after IV injection was calculated by the equation  $Cl = dose/ AUC$ . While,  $V_z$ , the apparent volume of distribution in the terminal phase, was calculated as  $V_z = \frac{dose}{\lambda z \times AUC}$ . Bioavailability (F) was calculated by dividing AUC obtained after SC administration by that determined following IV injection.

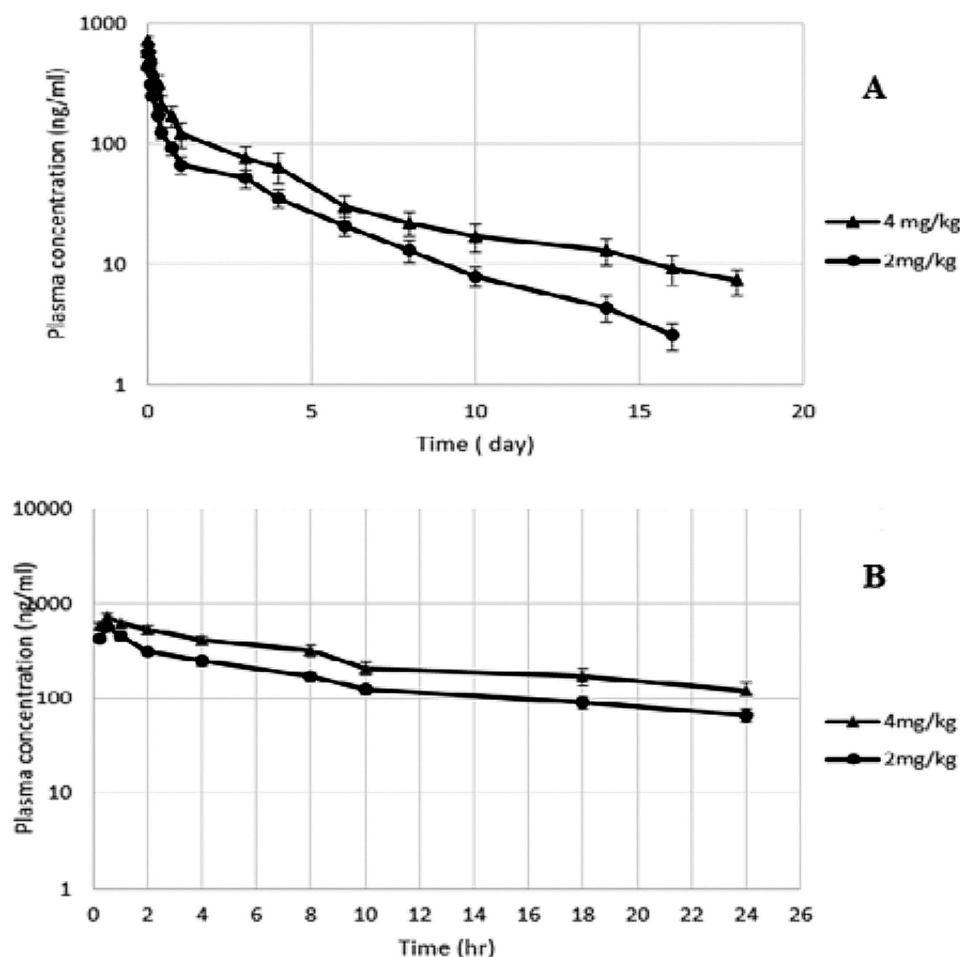
### Statistical analysis

All Data are presented as mean ± SEM. Arithmetic means were determined as appropriate, for each parameter and variable.

### Results

There were no noticeable adverse effects recorded in goats following IV and SC injections of tildipirosin (*no pain and swelling at the site of injection, no changes in the skin color, in addition, the pulse, respiratory rate, temperature, appetite and fecal consistency were normal*). The calibration curve was calculated by the linear regression equation  $y = 1805.5x - 51.71$ , where y represents the analytic area and x stands for the concentration (ng/ml). Linearity existed within range of 1 and 5400 ng/mL with a correlation coefficient of  $r^2 = 0.99$ . *Liquid chromatogram of blank plasma sample (without tildipirosin), tildipirosin standard (5400 ng/ml) and chromatogram of goat plasma sample at 4hr after SC injection of 4 mg/kg were shown in Figure 1 A, 1B& 1 C.*

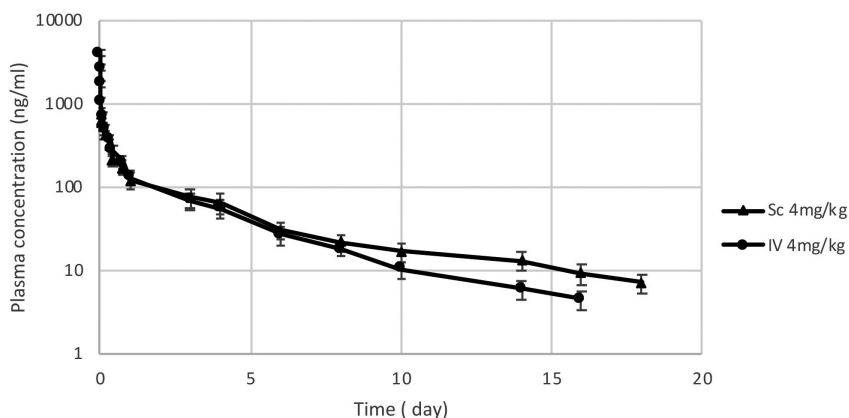
The plasma concentration-time curves of tildipirosin after each administration are depicted on a semilogarithmic plot in Figure 2 and Figure 3. After SC administration of tildipirosin at a single dose of 4 mg/kg, tildipirosin was detected



**Fig. 2** Plasma concentration-time profiles of tildipirosin in goats after Sc administration at 2 and 4 mg/kg. Values are expressed as mean  $\pm$  SEM (n=5). (A) showing the concentration-time during the whole process, (B) representing the concentration-time within 24 h. Values are expressed as mean  $\pm$  SEM (n=5).

in plasma up to the last collection time point 18 days after administration. While, it could be detected in plasma up to only 16 days after its SC and IV injections at a single dose of 2 and 4 mg/kg, respectively. The mean pharmacokinetic parameters following SC injection of tildipirosin at 2 and 4 mg/kg and IV administration of 4 mg/kg, and the absolute bioavailability (F%) are listed in Table 2. The  $C_{max}$  of tildipirosin were observed to be  $571.6 \pm 39.22$  and  $720 \pm 52.99$  ng/ml at 30 min after SC administration of 2 and 4 mg/kg, respectively. Like the  $C_{max}$ , the  $AUC_{0-last}$  values elevated in a dose-based manner and were calculated to be  $11.09 \pm 1.64$  and  $19.59 \pm 4.18$  after SC injection of tildipirosin at 2 and 4 mg/kg, respectively.

Meanwhile, after IV injection of tildipirosin at a single dose of 4 mg/kg, the  $AUC_{0-last}$  was  $20.27 \pm 3.38$   $\mu\text{g}\cdot\text{hr}/\text{ml}$ . The absolute bioavailability of tildipirosin was 96.64% following a single SC injection into the neck at a dose of 4 mg/kg. Tildipirosin was eliminated with elimination half-life values ( $T_{1/2 \lambda z}$ ) of  $75.34 \pm 2.71$  and  $94.76 \pm 8.13$  hr following SC injection at a dose 2 and 4 mg/kg, respectively, which is equivalent to 3.1 and 3.9 days. Moreover, after tildipirosin injection at 4 mg/kg intravenously, the  $T_{1/2 \lambda z}$  was determined to be 3.24 days. The MRT values after SC administration of tildipirosin at 2 and 4 mg/kg and following IV injection of 4 mg/kg were  $72.88 \pm 5.38$ ,  $86.14 \pm 7.29$ , and  $56.58 \pm 2.88$  hr, respectively. Following a single IV injection of



**Fig. 3** The plasma concentration-time curves of tildipirosin at a dose of 4 mg/kg after IV and Sc. Administrations. Values are expressed as mean  $\pm$  SEM (n=5).

tildipirosin at 4mg/kg, the  $V_z$  was  $24.69 \pm 4.10$  L/kg and the plasma clearance was  $0.216 \pm 0.029$  L/hr/kg.

## DISCUSSION

Tildipirosin, the most recent macrolide antibiotic, is characterized by possessing a prolonged, strong bacteriostatic activity, high bioavailability and drug levels especially in lung tissues and other unique properties<sup>11,16</sup>. Knowing the pharmacokinetic profile of any antibiotic is crucial to determine its appropriate dose regimen to maximize its efficacy and minimize the emergence of resistance pathogens and toxicity<sup>2-3,5,15</sup>. To the best of our knowledge, this is the first study on the pharmacokinetic feature of tildipirosin using different doses and routes of administrations in goats.

The findings of the current study showed that following a single SC injection of tildipirosin in goats at 4 mg/kg, the  $C_{max}$  (720 ng/ml) was similar to that previously published for cattle (711 ng)<sup>13</sup>. However, this  $C_{max}$  was comparatively slightly lower than that announced for swine injected intramuscularly with 4mg/kg (1000 ng/ml)<sup>11</sup> and for dogs injected intramuscularly with 4 mg/kg (1051 ng/ml). The  $C_{max}$  was achieved within short  $T_{max}$  (30 min) advocating its rapid absorption after SC injection. This result is parallel to that

reported by<sup>13</sup> who mentioned that tildipirosin was rapidly absorbed following SC injection in cattle as indicated by low value of  $T_{max}$  (23 min). Moreover, the present study revealed that the absolute bioavailability (F) of tildipirosin was 96.64% after SC administration in goats, which was higher than that reported in cattle (78.9%)<sup>13</sup>. This high bioavailability provides an evidence that tildipirosin is rapidly and almost completely absorbed following SC injection in goats.

Following IV injection of tildipirosin, the volume of distribution was 24.69 L/kg which is higher than that of other macrolides such as tilmicosin (15.3 L/kg)<sup>12</sup> and tulathromycin (11.0 L/kg)<sup>14</sup>. This high volume of distribution of tildipirosin is owing to its hydrophobic nature<sup>7</sup> and explaining its extensive diffusion in goat tissues. This finding is in accordance with<sup>16</sup> who declared that tildipirosin was widely distributed into porcine tissues as the concentration of the drug in the lung was 100 times higher than that in the plasma. Furthermore, the plasma clearance of tildipirosin was  $0.216 \pm 0.029$  L/hr/kg, which was lower than that revealed in cattle injected with tildipirosin at 4mg/kg by IV route ( $0.144$  L/hr/kg)<sup>13</sup>, and in dogs injected 2mg/kg tildipirosin intravenously<sup>21</sup> ( $0.72$  L/hr/kg) and for other macrolides including tilmicosin ( $0.686$  L/hr/kg)<sup>12</sup> and gamithromycin ( $0.712$  L/hr/kg)<sup>10</sup>, emphasizing the relatively higher plasma concentrations throughout the elimination phase.

The  $T_{1/2}$   $\lambda_z$  following SC injection of tildipirosin at a dose of 4 mg/kg was 94.76 hr which is equivalent to 3.9 ds. This value is comparable to that reported in dogs (3.7 days)<sup>21</sup>. However, it was shorter than that recorded in pigs (4.4 days)<sup>16</sup> and in cattle (8.5 days)<sup>13</sup>. This variation may be due to species difference. Dissimilarities in pharmacokinetic parameters are more commonly observed and linked to species variations, breed, age and/or the assay method used<sup>9</sup>.

Tildipirosin, similar to other macrolides, has been categorized as time- dependent killing antibiotic and hence the pharmacokinetic/ pharmacodynamic parameter time exceeding the minimum inhibitory concentration (MIC) ( $T > MIC$ ) is the preferred criterion to express its therapeutic effect. For instance, a prior investigation revealed that the  $MIC_{90}$  of tildipirosin for *Mannheimia haemolytic* and *Pasteurella multocida*, most commonly incorporated as a causative agent for bovine respiratory disease (BRD), was 1000ng/ml<sup>6</sup>. In the present study, the  $C_{max}$  of tildipirosin (720 ng/ml) in goat plasma following SC injection at 4 mg/kg was lower than the  $MIC_{90}$  for these bacteria. This may be attributable to the wide diffusion of the drug to the lung and this perspective is consistent with that of<sup>13</sup> who reported that although the  $C_{max}$ , in cattle injected subcutaneously with 4 mg/kg tildipirosin, was lower than the  $MIC_{90s}$  (1000 ng/ml) against most pathogens causing BRD, the mean lung concentration was multifold higher than the plasma concentration and exceeding that  $MIC_{90}$  for 16 days. Therefore, the plasma concentration cannot provide guidance for dosing regimen and this highlight the need for further study to investigate the concentration of tildipirosin in various tissues particularly the lung. Furthermore, further investigation is essential for more clarification of the pharmacokinetic/ pharmacodynamic relationship of tildipirosin to recommend suitable doses and dosing frequencies in goats.

In conclusion, The SC injection of tildipirosin

in goats revealed rapid absorption with high bioavailability, extensive distribution, and long persistence in the body. Knowing the pharmacokinetic characters of tildipirosin could afford guidance for its utilization in goats.

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