



RESEARCH ARTICLE

Pharmacokinetics of a New Parenteral Formulation of Tilmicosin-La in Cows

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ABSTRACT

The blood serum pharmacokinetics of a Poloxamer-407 based new pharmaceutical preparation of 39% tilmicosin was determined in cows. Two dose levels injected SC, were assessed: 10 mg/kg (Til-LA₁₀) and 26 mg/kg (Til-LA₂₆). Tilmicosin was determined using HPLC, also electrocardiographic (EKG) monitoring in all animals before and at 1, 2 and 4 h after the injection of the drug was performed to measure key EKG parameters, including heart rate. Maximum serum concentration values were 2.6 µg/mL and 1.23 µg/mL, occurring 4.9 and 5.1 h after the injection of Til-LA₂₆ and Til-LA₁₀, respectively. Mean residence time was statistically larger for Til-LA₂₆ (50.4±5.8 h) than Til-LA₁₀ (37.4±4.7 h) (P<0.05), with T_{1/2el} of 39.8 h for Til-LA₂₆ and 33.2 h for Til-LA₁₀. There were no differences in relative bioavailability as adjusted for dose (AUMC of 8663 µg/mL·h² for Til-LA₂₆ and 2858 µg/mL·h² for Til-LA₁₀). This new formulation as dosed for Til-LA₂₆ possesses a long T_{1/2el} of tilmicosin and resulted in a lack of changes in the EKG and heart rate. Based on PK/PD ratios tilmicosin is regarded as a time-dependent antibacterial drug. Considering a theoretical minimum therapeutic serum concentration (MTC) of 0.1 µg/mL useful concentrations can be achieved for up to 192 h with Til-LA₂₆ and an AUC/MTC ratio of 1514 without cardiac toxicity. Further studies are necessary to correlate PK/PD parameters obtained with clinical efficacy and a more thorough analysis of cardiac toxicity is required to determine the suitability of this preparation in bovine medicine.

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INTRODUCTION

Tilmicosin, a semi-synthetic macrolide antibacterial drug shows adequate pharmacokinetic features to treat respiratory bacterial diseases of bovines and has also been used for parenteral dry-cow therapy (Dingwell *et al.*, 2002). Tilmicosin has outstanding tissue diffusion to respiratory tissues and mammary gland (Scott 1995; Brumbaugh *et al.*, 2002; Lombardi *et al.*, 2011; Soliman and Ayad, 2014). Due to its large apparent volume of distribution, useful serum concentrations have been considered to be at or above 0.1 µg/mL (Ramadan, 1997). Reference pharmaceutical preparation (Micotil®, Elanco Animal Health, Mexico) has a half-life of approximately 30 h (Ziv *et al.*, 1995; Lombardi *et al.*, 2011). These features explain the resolution of a reasonable amount of respiratory infections with a single injection of 10 mg/kg (Scott, 1995; Soliman and Ayad, 2014). Also, tilmicosin

has been appointed as treatment for *Staphylococcus* spp. induced mastitis during the dry-cow period (Owens *et al.*, 1999). Nevertheless, a certain number of cases need a second dose to achieve bacteriological and clinical cure. To minimize handling of animals, it is here postulated that the pharmacokinetics/pharmacodynamic (PK/PD) ratios of the drug, as a time-dependent antibacterial agent (Ziv *et al.*, 1995; Lombardi *et al.*, 2011), can be optimized by further extending its mean residence time. Such a manoeuvre will also take advantage of the anti-inflammatory effects of tilmicosin (Buret, 2010; Munic *et al.*, 2011), and of its ability to stimulate phagocytosis (Brumbaugh *et al.*, 2002; Shinkai, 2008; Buret, 2010). Tilmicosin, as prepared in Micotil®, is based on propylene glycol to achieve useful serum concentrations for 48 to 72 h, setting minimum therapeutic concentrations (MTC) at 0.1 µg/mL (Ziv *et al.*, 1995; Ramadan, 1997). The experimental preparation of

tilmicosin here proposed contains the hydrogel Poloxamer 407, a vehicle that allows a long acting effect of some drugs (Brayden, 2000; Sun *et al.*, 2004). Poloxamer-407 has thermosensitive characteristics that produce hydrogel-based formulations with flow at low room temperature and will form a gel-like matrix with minimum inflammation at body temperature (Hoffman, 2002). Hence, this paper describes the pharmacokinetics (PK) of a new preparation of tilmicosin injected subcutaneously at two dose levels: 10 mg/kg (Til-LA₁₀), and 26 mg/kg (Til-LA₂₆).

MATERIALS AND METHODS

Study design and animal handling complied with Mexican prescripts (NOM-062-ZOO-2001). This trial was implemented at a dairy farm under intensive production with Holstein/Friesian cows. Twenty, two to five years old, treatment-free cows, with a mean weight of 545±12 kg and entering the dry-cow period were randomly divided in two groups of 10 cows each: group Til-LA₁₀, treated with a single SC injection of tilmicosin (10 mg/kg; 1 mL/39 kg of body weight) utilizing the experimental preparation of 39% tilmicosin phosphate (Patent 212148; *Instituto Mexicano de la Protección Industrial*, Mexico City); and group Til-LA₂₆ cows injected with the same preparation but at a dose of 26 mg/kg (1 mL/15 kg of body weight). No more than 10 mL/injection site were administered at either side of the neck region with 16 gauge needles, 3 cm long. Injection was directed downwards, while pulling the skin; to allow the formation of a cavity where the drug preparation was deposited. Intravenous injection of tilmicosin is not recommended due to acute cardiac toxicity.

Blood samples were obtained by direct jugular puncture with Vacutainer tubes before injection and at 0, 0.5, 1, 2, 6, 8, 10, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180, 192, 204 h post-injection. Serum was obtained by centrifugation (3000 g for 15 min), samples labelled and stored at -20°C until analyzed. Tilmicosin was determined through high-performance liquid chromatography (HPLC), as reported by Parker and Patel (1994). Based on Akaike's criterion (Olofsen and Dahan, 2014) and with computerized curve-stripping program (PKAnalyst®; Micromath Scientific Software, SLM, USA) best fitting was obtained with a two compartment model with first-order input and first-order output (Model 13, $r \geq 0.98$), whose formula is:

$$\text{Concentration (Time)} = Ae^{-\alpha \cdot \text{Time}} + Be^{-\beta \cdot \text{Time}} + Ce^{-K_{AB} \cdot \text{Time}}$$

Pharmacokinetic variables obtained were: $T_{1/2el}$ =elimination half-life; $T_{1/2ab}$ =absorption half-life; C_{MAX} =maximum serum concentration; T_{MAX} =time to reach C_{MAX} ; AUC=area under the concentration-time curve; AUMC=area under the first moment of the concentration-time curve; MRT=mean residence time; Fr=relative bioavailability of Til-LA₁₀ vs Til-LA₂₆, corrected for dose [$Fr = (AUC_{Til-LA_{26}} \cdot \text{Til-LA}_{10} \text{ dose}) \div (AUC_{Til-LA_{10}} \cdot \text{Til-LA}_{26} \text{ dose}) \times 100$]. Also the following PK/PD ratios were obtained: AUC/MTC (MTC=minimum arbitrary therapeutic concentrations so far accepted i.e., 0.1 µg/mL), and $T > MTC$. Descriptive statistical data for these parameters was calculated using

SPSS 11.0 (SPSS Inc., Chicago, IL). Significant differences ($P < 0.05$) between Til-LA₁₀ and Til-LA₂₆ parameters was assessed with Wilcoxon rank sum test and Student's *t*-test.

Possible cardio-toxicity of tilmicosin was studied with electrocardiography (ECG), obtained with an Edan iM8B Vet AG30 apparatus (EDAN Instruments, Inc., Nanshan - Shenzhen, China). Cows were restrained and placed on a thick plastic sheet. Needle electrodes were placed in forelegs in the anterolateral skin fold immediately above the olecranon process and in the skin fold at knee level in rear legs to obtain recordings at DI, DII and DIII derivations. Parameters studied were: heartbeat rate and cardiac rhythm as well as amplitude, morphology and duration of P and T waves, QRS complex, duration of P-R, Q-T and R-R intervals, the S-T segment and the electrical axis of the heart, as described by Macfarlane *et al.* (2010). Tracings were obtained before the injection of either tilmicosin preparation and 1, 2 and 4 h after. Significant differences were studied before and after injection of tilmicosin and differences analyzed through variance and Student's *t*-tests.

After 30 days from injection, all animals were slaughtered as stipulated by Mexican regulations (NOM-0033-ZOO-1995), injection sites dissected, macroscopically inspected for lesions and processed for routine histopathology.

RESULTS

Mean±SD serum concentrations vs time profiles of Til-LA₁₀ and Til-LA₂₆ after a single SC injection of either 10 or 26 mg/kg, respectively, are presented in Fig. 1. Table 1 shows mean±SD values of the pharmacokinetic parameters obtained, as well as their statistical comparison.

Maximum serum concentration (C_{MAX}) was 1.23±0.09 µg/mL for group Til-LA₁₀ and 2.6±0.13 µg/mL for group Til-LA₂₆. Terminal phase half-lives ($T_{1/2el}$) were 33.2±5.1 h and 39.8±4.5 h, respectively. Mean residence time was slightly larger for Til-LA₂₆, as compared to Til-LA₁₀ (50.4±5.8 h vs 37.4±4.7 h) ($P > 0.05$). Relative bioavailability (Fr) of the Til-LA₂₆ group, as compared to Til-LA₁₀, corrected for dose level, was negligible (98% for Til-LA₂₆). Absorption half-life ($T_{1/2ab}$) was also very similar between groups (0.90 h vs 0.91 h for Til-LA₁₀ and Til-LA₂₆, respectively). However C_{MAX} was higher in the Til-LA₂₆ as compared to Til-LA₁₀ (2.6 µg/mL vs 1.23 µg/mL); yet, T_{MAX} was not statistically different.

All cows from both groups remained clinically healthy throughout the study and no signs of visible pain were detected. However, a clearly distinctive bulging response in the injection sites was observed in both groups. This bulge was more an occupying mass than an inflammation. It disappeared completely after 72 h in both groups. Histopathological analysis of injection sites showed neither macroscopic signs of inflammation nor of scar tissue.

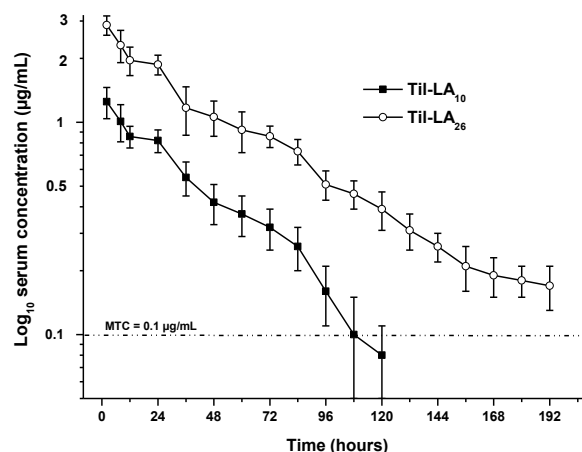
All ECG tracings were noticeably flat as expected for this species and basal values showed lack of stress as these animals were accustomed to daily handling. Morphologies of the P wave, QRS complex, and T wave, revealed no apparent changes induced by either preparation of tilmicosin. P waves were, either positive or negative with some isoelectrical tracings. Also, duration of P and T waves, of the QRS complex, and of the Q-T, P-R, and R-R intervals showed no differences between

groups, neither before nor after the injection of either preparation of tilmicosin. Also, as expected, the S-T segment was particularly long, with T waves showing variable morphology. Yet such morphologies were unrelated to injection of either Til-LA₁₀ or Til-LA₂₆. Similarly rS configuration did not show significant differences neither before nor after injection of tilmicosin. Basal cardiac rates in the Til-LA₁₀ and the Til-LA₂₆ groups were 87.6±6.5 and 86.8±5.5, respectively and remained almost unchanged after the three posterior ECG readings.

DISCUSSION

Microbiological determination of tilmicosin concentrations in biological samples reveals only 20-25% of the actual concentration of the drug (Stobba-Wiley *et al.*, 2000). Hence, HPLC analysis was chosen as analytical technique and it can be safely assume that the fraction detected is the relevant analyte to assess biological activity (Lombardi *et al.*, 2011) and can be safely utilized to calculate the pharmacokinetic behaviour of the active fraction of the drug.

Cardiotoxicity of tilmicosin precluded the determination of PK of the drug after IV administration. Consequently, neither absolute bioavailability nor apparent volume of distribution values were determined. Also, the tissue irritation capability of this macrolide drug impeded PK studies after IM injection. This study compares the PK of two dose levels of an experimental preparation of tilmicosin. Setting MTC at 0.1 µg/ml, based on data derived from other studies (Ziv *et al.*, 1995; Ramadan, 1997) and considering that tilmicosin is distributed outside the central compartment at least 10-20 times (Helton-Groce *et al.*, 1993; Modric *et al.*, 1998; Soliman and Ayad, 2014), the time period with serum concentrations of tilmicosin at or above a therapeutic level, was statistically different between Til-LA₂₆ (192 h) and Til-LA₁₀ (120 h) ($P < 0.001$). Also, AUC/MTC values were higher in the Til-LA₂₆ group, as compared to the Til-LA₁₀ group (1514 and 592) ($P < 0.01$). Figure 1 shows this time-extension of useful serum concentrations of tilmicosin. In contrast, Lombardi *et al.* (2011) demonstrated that when the dose of a standard preparation of tilmicosin was increased from 10 to 20 mg/kg in light or heavy beef calves, proportional increase in both AUC and C_{MAX} were obtained. However, the MTC (0.1 µg/ml) did not surpassed 72 h with either dose. It is likely to assume that Poloxamer-407 in the experimental preparation increases time above MTC, allowing an initial priming absorption lacking cardiac toxicity. Correspondingly MRT for Til-LA₂₆ was 50.4±5.8 h and 37.4±4.7 h for Til-LA₁₀; and $T_{1/2el}$ values were roundly 39.8 h vs 33.2 h for Til-LA₂₆ and Til-LA₁₀, respectively. These values are both higher than the ones obtained in former studies using the standard preparation of tilmicosin ($T_{1/2el}$ =30 h) (Modric *et al.*, 1998). Poloxamer-407 improves solubility of some drugs, reduces their hydrolytic degradation and provokes their controlled release, often resulting in improved bioavailability (Vargas *et al.*, 2008). Poloxamer-407 as thermo-reversible vehicle becomes gel at body temperature (37-40°C), entrapping tilmicosin which is then release in a sustained manner while allowing an initial priming absorption.



MTC = minimum accepted serum therapeutic concentration.

Fig. 1: Mean±SD plasma concentrations vs time profiles of tilmicosin in Holstein/Friesian cows after the SC injection of an experimental, poloxamer 407-based preparation of tilmicosin (390 mg/ml), intended for extended long action, at two dose levels 10 mg/kg (Til-LA₁₀) and 26 mg/kg (Til-LA₂₆).

Table 1: Pharmacokinetic values and PK/PD ratios of tilmicosin in Holstein/Friesian cows from a new long-acting preparation of tilmicosin administered, subcutaneously, at two doses: 10 mg/kg (Til-LA₁₀) or 26 mg/kg (Til-LA₂₆)

Pharmacokinetic variable	Til-LA ₁₀	Til-LA ₂₆
$T_{1/2el}$ (h)	33.2±5.1a	39.8±4.5b
$T_{1/2ab}$ (h)	0.90±0.05a	0.91±0.04a
C_{MAX} (µg/mL)	1.23±0.09a	2.6±0.13b
T_{MAX} (h)	5.1±0.4a	4.9±0.4a
AUC (µg/mL·h)	59.2±3.8a	151.4±8.4b
AUMC (µg/mL·h ²)	2858±17.4a	8663±21.1b
MRT (h)	37.4±4.7a	50.4±5.8b
Fr %	-	98
AUC/MTC	592a	1514b
T>MTC (h)	120a	192b

Values (Mean±SD) with different letter in a row differ significant ($P < 0.05$). $n=10$ cows/group; $T_{1/2el}$ =elimination half-life; $T_{1/2ab}$ =absorption half-life; C_{MAX} =maximum serum concentration; T_{MAX} =time to reach C_{MAX} ; AUC=area under the concentration-time curve; AUMC=area under the first moment of the concentration-time curve; MRT=mean residence time; Fr=relative bioavailability of Til-LA_r vs Til-LA_{exp}, corrected for dose [$Fr=(AUC_{Til-LA_{26}} \cdot Til-LA_{10} \text{ dose}) \div (AUC_{Til-LA_{10}} \cdot Til-LA_{26} \text{ dose}) \times 100$]. AUC/MTC (MTC=minimum accepted therapeutic serum concentration, taken in this study as 0.1 µg/ml) and T>MTC=PK/PD ratios.

Presently, the time mark of 192 h to reach MTC merits further work by examining the concentrations of the drug in lungs and/or in mammary tissue and milk. Notwithstanding the above, advantages of injecting Til-LA₁₀ or Til-LA₂₆ will include a reduction in work-load if long treatment schemes are required. Also potentially improved clinical outcome can be expected based on AUC/MTC ratios obtained (592 and 1514 for Til-LA₁₀ and Til-LA₂₆, respectively), a key feature required for macrolide antibacterial drugs (Burch, 2010). This requires confirmation under various clinical settings *i.e.*, parenteral dry-cow therapy.

Tilmicosin is extensively distributed outside the central compartment into respiratory tissues (Soliman *et al.*, 2014), mammary gland (Helton-Groce., 1993) and even at the intracellular level (Brumbaugh *et al.*, 2002), and for longer time, as compared to serum concentrations (Ibrahim, 2011). Consequently, a low MTC has been accepted (0.1 µg/mL) (Ziv *et al.*, 1995; Ramadan, 1997; Ibrhim, 2011). Tissue concentrations of tilmicosin were

not determined in this trial. Yet, it is tempting to speculate that clinical efficacy for respiratory and mammary infections could be improved with Til-LA₂₆ due to its long residence time and assuming a similar behaviour of tilmicosin once it is released from Poloxamer-407. Nevertheless, tissue concentrations of tilmicosin as well as efficacy studies are warranted.

All cows from both groups remained clinically healthy throughout the study without signs of visible pain, even in Til-LA₂₆ cows. Electrocardiographic findings and cardiac rates were very much in agreement with set standards (Macfarlane *et al.*, 2010) and were not different when comparing either, both groups or data before and after administration of either dose of tilmicosin. Tracings obtained tended to be flatter compared to classical ECG ones in other species. This phenomenon has been attributed to the diffuse organization of Purkinje fibers in the bovine myocardium. Yet, the ECG in cows can be useful to differentiate arrhythmias; being sinus arrhythmias, the most common dysrhythmias observed when tilmicosin is overdosed (Macfarlane, 2010). However in the 20 cows studied, no sinus arrhythmias was detected. Mean frontal plane electrical axis of the QRS complex varied from 79° to 275°, and did not change substantially after the injection of either preparation of tilmicosin. Although these results are encouraging for low cardiac toxicity of the experimental formulation tested, further studies also measuring blood creatine phosphokinase are necessary. Also, it is mandatory to determine safety of this pharmaceutical design in all types of cattle i.e., debilitated animals as well as, drug residues of the drug and metabolites before this preparation can be regarded as potentially useful in bovine medicine.

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Author's contribution: LG and HS conceived and designed the study. IM-C and RS executed the experiment and JM-C analyzed the sera samples.

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