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Pharmacokinetic behavior of marbofloxacin after intravenous, subcutaneous and intramuscular administrations in llamas (*Lama glama*)

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ABSTRACT

The purpose of this study was to determine the pharmacokinetic profile of marbofloxacin in llamas following single IV, IM and SC administration of 5 mg/kg bw. To assess whether these routes could be an option for the administration of marbofloxacin in this specie and estimate efficacy predictors (PK/PD) from bibliographic MIC_{90} values. The principal pharmacokinetic parameters were $V_{ss} = 0.72 \pm 0.22$ L/kg, Cl = 0.09 ± 0.03 L/kg h (for IV administration), $C_{max} = 7.43 \pm 1.28$ μg/mL and 6.94 ± 2.19 μg/mL for IM and SC administration, respectively; $t_{1/2\lambda} = 9.16 \pm 1.08$ h, 8.47 ± 0.31 h and 6.26 ± 0.87 h and MRT = 7.30 ± 1.07 h, 8.21 ± 1.57 h and 8.27 ± 0.96 h for IV, IM and SC administrations, respectively. The values obtained for the pharmacokinetic parameters were not significantly different among routes of administration except C_{max}, V_z and $t_{1/2\lambda}$. The values obtained from the PK/PD indices were C_{max}/MIC_{90} (12.4–32.3) and AUC_{0-24}/MIC_{90} (105.7–178.52 h); taking into account these values, it was concluded that a IM or SC dose of 5 mg/(kg day) of marbofloxacin would be adequate in treating infections caused by susceptible bacteria with MIC < 0.44 μg/mL.

1. Introduction

South American Camelids (SAC) are a rapidly growing segment of small ruminant livestock in South America and the US and they are the only economic resources in vast areas of natural pastures of the highlands, near the Andes, where agriculture or the raising of other domestic animal species is not possible (Pinto Jiménez et al., 2010). They are raised for their prized fibers and meat, which has extraordinary nutritional value and low cholesterol content (Vargas-Terán, 2005).

Bacterial diseases of the reproductive, gastrointestinal and respiratory systems are common problems in llamas and these pathologies are a significant problem and usually represent an economic disaster for the owner (Whitehead, 2009).

Although camelids are not taxonomically classified as ruminants, but tylopodus (Tylopoda), they are functional ruminants (Fowler and Zinkl, 1989), hence sometime the clinicians extrapolate the dosage from ruminant species.

Because of the differences in the kinetic behavior of many drugs in different species, pharmacokinetic studies are necessary in order to design rational therapeutic dosage schedules for the llamas and thus, avoid subtherapeutic or toxic levels, bacterial resistance and a possible risk of unacceptable residues in edible tissues.

However, data on the pharmacokinetics of fluoroquinolones in camels, llama and alpaca are limited (Gavrielli et al., 1995; Christensen et al., 1996; Aliabadi et al., 2003; Gandolf et al., 2005; Laraje et al., 2006).

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Marbofloxacin has a broad microbial spectrum activity and pharmacokinetic profile which suggests good tissue penetration making this drug a suitable second choice treatment especially for intestinal and pulmonary infections.

The purpose of this study was to determine the pharmacokinetic profile of marbofloxacin in llamas following single IV, IM and SC administration to assess whether these routes differ among each other in this specie and estimate efficacy predictors (PK/PD) from bibliographic MIC_{90} values.

2. Materials and methods

2.1. Animals

Six clinically healthy llamas (1.5–6 years old; 146.8 ± 25.38 kg) were used. The study was approved by the Animal Experimentation Ethics Committee of the School of Veterinary Medicine of the Universidad Católica de Córdoba, Argentina.

2.2. Experimental design

The study followed a three-part crossover with a wash period of 15 days between the three phases.

Each group received a 5 mg/kg BW dose using a 10% aqueous solution of marbofloxacin (Marbocyl®, Vetoquinol, Lure, France). One group received the dose intravenously (IV) into the right jugular vein, another group intramuscularly (IM) into the semitendinous muscle and the remaining group subcutaneously (SC) in the dorsal lumbar region. The volume injected was 7.34 ± 1.26 mL (range 5.75–8.95).

Five milliliter blood samples were collected with an 18G catheter placed in the left jugular vein, at 0, 5 (only IV), 10, 15 (only IV), 30, 45 min, and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 30, 36 and 48 h after administration. After 30 min of collection, the samples were centrifuged at 1800 × *g* for 20 min. Serum aliquots were frozen (−80 °C) until assayed with analyses performed within 4 weeks after sample collection.

2.3. Analytical assay

Serum marbofloxacin concentrations were quantified using HPLC/UV according to a method previously described by Waxman et al. (2001). Marbofloxacin was provided by Vetoquinol (Lure, France) and ofloxacin was used as an internal standard (Sigma Chemical Co., St Louis, MO, USA). Separation was achieved with a 150 mm × 4.6 mm, 5 μm Kromasil® 100 C18 column, and a 30 mm × 4.0 mm Kromasil® 100 C-18 5 μm guard column, both operated at room temperature. The mobile phase consisted of buffer pH 2.7–methanol–acetonitrile–acetic acid–triethylamine (74:20:4:1:1, v/v/v/v/v). The buffer pH 2.7 was a 0.4% aqueous solution of tetrabutylammonium hydrogen sulphate (p/v) and diammonium hydrogen phosphate (p/v). The UV detection wavelength was 295 nm and the flow rate was 0.6 mL/min.

The quantification limit was 0.025 μg/mL and the method was linear between 0.025 and 15 μg/mL. Precision and accuracy of the limit of quantification were 6.55 ± 2.12% and 119.01 ± 1.22%, respectively. The inter-assay and intra-assay reproducibility were 5.97 ± 1.41% and 5.71 ± 3.9%, respectively. The mean percentage recoveries of marbofloxacin from serum samples were 91.73 ± 4.57%.

2.4. Sample processing

A volume of 300 μL of serum was placed into 15 mL screw capped tubes, and 75 μL of the internal standard solution (ofloxacin, 5 μg/mL in formic acid 0.1 N) and 4.5 mL of trichloromethane were added. After being agitated for 10 min in a horizontal agitator, the samples were centrifuged at 3200 × *g* for 7 min at 10 °C and the organic layer was aspirated and transferred to another tube then evaporated under nitrogen stream at 40 °C. The samples were then re-dissolved in 150 μL of mobile phase and a 20 μL aliquot from the solution was injected into the HPLC system.

2.5. Pharmacokinetic analysis

Marbofloxacin serum concentrations vs time curves were subsequently processed by the pharmacokinetic PCnolin V4.0 software package (Statistical Consultants INC, Lexington, KY, USA). The non-compartmental pharmacokinetic parameters of elimination rate constant (λ, calculated as the slope of the terminal phase of the plasma concentration curve that included a minimum of four points), half-life ($t_{1/2\lambda}$, where $t_{1/2\lambda} = 0.693/\lambda$), area under the plasma concentration vs time curve (AUC) (calculated by logarithmic trapezoidal rule for IV route and for IM and SC routes were calculated using a combination of linear trapezoidal rule in the ascending part of the curve and logarithmic trapezoidal rule in the descending phase), area under the first moment curve (AUMC); mean residence time (MRT, where MRT = AUMC/AUC), and clearance (where Cl = Dose iv/AUC) and volume of distribution (where $V_z = \text{Dose}/\lambda_z \cdot \text{AUC}$) were calculated based on moment methods. Also, the IV concentrations were subjected to compartmental analysis using non-linear least squares regression analysis. The data were weighted with the inverse of the squared fitted value. Polyexponential equations were fitted to the plasma concentration–time curves and the number of exponential terms was determined by the application of Akaike's Information Criterion (AIC), residual sum of squares (R_s) and the analysis of the residual's plots. The elimination half-life ($t_{1/2\beta}$) was calculated by $t_{1/2\beta} = 0.693/\beta$. The relative bioavailability (F) of marbofloxacin was calculated using the following formula:

$$F(\%) = \left(\frac{\text{AUC}_{\infty(\text{e.v.})}}{\text{AUC}_{\infty(\text{i.v.})}}\right) \times 100$$

In this formula, $\text{AUC}_{\infty(\text{e.v.})}$ represents the area under the concentration–time curve from zero up to infinite time when marbofloxacin was extravascularly (IM or SC) administered. Furthermore, $\text{AUC}_{\infty(\text{i.v.})}$ represents the area where marbofloxacin was injected intravascularly.

All values are presented as mean ± S.D.

2.6. PK/PD indices

The peak serum concentration (C_{max}) and area under the concentration-time curve from 0 to infinite (AUC_∞) were applied in the calculation of the predictors of efficacy for concentration dependent antibiotics: C_{max}/MIC (C_0/MIC for IV administration, C_0 = observed values) and AUC_∞/MIC for all administration routes (AUC and C_{max} values were from a non-compartmental model). Mean MIC_{90} values of isolates from respiratory infections were used from published data (Meunier et al., 2004).

2.7. Statistical analysis

Normal distributions of samples were evaluated using a Shapiro–Wilk test. Differences between PK parameters of treatment groups were determined using a two-way ANOVA and Bonferroni test, and *T*-paired for differences between plasma level CK. A value of $P < 0.05$ was considered significant.

3. Results

Signs of pain or lameness were not observed after IM or SC administration of marbofloxacin in llamas. No local or systemic adverse reaction to marbofloxacin occurred after IV, IM or SC injection. The only exception was a temporal elevation of creatine kinase (CK) at 12 and 24 h after administration, with significant differences (Table 1). Also, we calculated the quantity of damage tisular from extrapolated values (taking into account the basal CK level, and an extrapolated muscle activity of 4300 UI/g (Toutain et al., 1995).

Serum marbofloxacin concentration–time curves obtained after IV, IM and SC administration are shown in Fig. 1. Data collected after IV administrations were fitted to a two-compartment open model. Pharmacokinetic