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Pharmacokinetic/pharmacodynamic analysis by Monte Carlo simulation of cefquinome in llamas, following intravenous, intramuscular and subcutaneous administration in serum and tissue cage fluid

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ABSTRACT

The objective of this study was to determine the pharmacokinetic behavior of cefquinome (CFQ) in serum and tissue cage fluid (TCF) in llamas, after intravenous (IV), intramuscular (IM) and subcutaneous (SC) administration of 2 mg/kg. Two tissue cages (TC) per animal were implanted in the subcutaneous tissue, on the *pectoralis major* muscles area. Serum and TCF concentrations of CFQ were determined by microbiological assay using *Klebsiella pneumoniae* ATCC 10031 as the test microorganism. Minimum inhibitory concentrations (MIC) of CFQ against *Escherichia coli* and *Staphylococcus aureus* strains isolated from llamas were determined by broth microdilution method. *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 strains were used as controls. Finally, a pharmacokinetic/pharmacodynamic (PK/PD) analysis by Monte Carlo simulation, based on pharmacokinetic data obtained from IV, IM and SC administration of CFQ in llamas and MIC values, was carried out to evaluate the antimicrobial efficacy of the dose regimen. CFQ showed a limited distribution, with values of Vdss = 0.10 ± 0.01 and Vz = 0.18 ± 0.04L/kg. Total body clearance (0.05 ± 0.01 L*kg/h) was lower than reported in other species. Absolute bioavailability of IM and SC routes was complete (124 ± 45% and 94 ± 35%, respectively). Moreover, CFQ presented a AUC_{TCF}/AUC_{serum} ratio <1 after IV administration, but ≈2 after IM and SC routes and showed a higher permanence in TCF compared with serum. These findings could determine higher T>MIC values, resulting in a better antimicrobial efficacy of CFQ in this biological compartment. MIC_{90} values for *E. coli* and *S. aureus* were 0.06 μg/mL and 0.50 μg/mL, respectively, with a MIC_{90}/MIC_{50} = 2. Based on the PK/PD analysis, a dose regimen of CFQ of 2 mg/kg q12 h by IV, IM and SC routes, could be effective for the treatment of infections caused by pathogens with MIC values ≤0.50 μg/mL. Therefore, CFQ appears to be a good choice against *E. coli* and *S. aureus* in llamas.

1. Introduction

Cefquinome (CFQ) is a fourth-generation cephalosporin used exclusively in veterinary medicine with a broad antimicrobial spectrum and high stability against β-lactamases produced by many clinically important bacteria. CFQ is approved for the treatment of respiratory tract infections in cattle and pigs, acute mastitis and foot rot in cattle, calf septicemia and metritis–mastitis–agalactia syndrome in sows. It has been used to prevent severe pneumonia in piglets (caused by *Actinobacillus pleuropneumoniae, Klebsiella pneumoniae*, and *Streptococcus pneumoniae*) (EMEA, 2009; Zhang et al., 2014). Also, several authors recommend the use of CFQ in the treatment of cattle diarrhea (Constable, 2004, 2009; Constable et al., 2008; Thomas et al., 2006).

Pharmacokinetics of CFQ was studied in dogs (Limbert et al., 1991), pigs (Limbert et al., 1991; Lang et al., 2002; Li et al., 2008; Zhang et al., 2014), calves (Limbert et al., 1991), horses (Parlevliet et al., 2009; Winther et al., 2011), buffaloes (Dinakaran et al., 2013),

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sheep, goats (Dumka et al., 2013;) and camels (Al-Taher, 2010), but to our knowledge, there are no pharmacokinetic studies of CFQ in llamas.

South American Camelids are a source of fiber, meat, work and other products in disadvantaged areas of South America, allowing the sustainable economic development in this region. *Staphylococcus aureus* is a major pathogen in llamas, and is frequently isolated from skin and wounds caused by thorns, bites and trauma. *S. aureus* was also isolated from animals with keratoconjunctivitis (Brightman et al., 1981; Fowler, 2010). Moreover, *Escherichia coli* may be related to different pathologies such as meningoencephalitis, diarrhea, and reproductive loss (Thedford and Johnson, 1989; Frank et al., 1998; Rulofson et al., 2001; Mercado et al., 2004; Tibary et al., 2006; Whitehead and Anderson, 2006; Whitehead, 2009; Fowler, 2010). Tissue cage models (TCM) were included in pharmacokinetic studies in pigs (Ding et al., 2010; Zhang et al., 2014), horses (Voermans et al., 2006), calves (Greko et al., 2002; Greko et al., 2003), rabbits (Rule et al., 2010), camels (Aliabadi et al., 2003), dogs (Walker et al., 1990), goats (Aliabadi and Lees, 2001), cats (Pelligand et al., 2011) and yellow cattle (Shan et al., 2014).

TCM may be a useful tool to evaluate the exposition kinetics of the microorganism to the antimicrobial in the interstitial fluid. The implementation of the TCM is a minimally invasive technique and does not involve the slaughter of animals or pain produced by a series of samples for biopsy (Ziv et al., 1982).

The pharmacokinetic/pharmacodynamic (PK/PD) approach is an useful tool in the selection of dosage regimens of antimicrobial agents, allowing for its rational use, maximizing the antimicrobial efficacy, minimizing the emergence of resistance and its further spreading to animal and human population to promote the prudent use of antimicrobials (Toutain et al., 2002; Ungemach et al., 2006; Papich, 2014). PK/PD parameters are surrogate markers commonly used to predict therapeutic efficacy and prevention of antimicrobial resistance, and expresses exposition relationships of the microorganism to the antimicrobial (Toutain et al., 2002; Papich, 2014).

In PK/PD analysis, the introduction of inter-individual pharmacokinetic variability and the population-pharmacodynamic data is essential in determining the antimicrobial efficacy of a dose regimen in a patient population. The Monte Carlo simulation is a useful tool to provide assistance in the optimization of empirical antimicrobial therapies, simulating the pharmacokinetic and pharmacodynamic variability (Drusano, 2003, 2004; Asín-Prieto et al., 2015).

The objectives of this study were: (1) to evaluate the pharmacokinetic behavior of CFQ in llamas after an intravenous (IV), intramuscular (IM) and subcutaneous (SC) administration of 2 mg/kg in serum and tissue cage fluid; (2) to determine the minimum inhibitory concentration (MIC) of CFQ against regional *S. aureus* and *E. coli* strains isolated from llamas, and (3) to perform a PK/PD analysis by Monte Carlo simulation to evaluate the efficacy of a dose regimen of CFQ (2 mg/kg) administered by IV, IM and SC routes against pathogens in serum and tissue cage fluid.

2. Materials and methods

2.1. Selection of animals, tissue cage manufacturing, implantation and treatments

This research was approved by the Commission of Bioethics and Animal Welfare of the Faculty of Agricultural Sciences of Catholic University of Córdoba (CBBA.07.2013.UCC). Six healthy, castrated, male llamas were included in this study. The animals aged between 2 ± 0.5 years and weighed 113 ± 20.5 kg. Animals were not exposed to any drug treatment 2 months previous to the experiment, and received food and water *ad libitum*.

Tissue cages (TC) were made of polivynyl chloride, tubular shaped, with an outer measure of 9.24 cm long and 2.20 cm of internal diameter. It was perforated with 20 holes of 0.89 cm of diameter, equally spaced with lineal disposition in 5 rows of 4 holes each. Two TC per animal were implanted in the subcutaneous tissue on the superficial pectoral muscles area, according to the technique of Bengtsson et al. (1984). The surgical technique was performed under deep sedation with xylazine (Xylazine® 20% Richmond Vet Pharma, Argentina), at a dose of 0.01 mg/kg IV and local infiltration anesthesia with lidocaine (Lidocaine® 2%, Richmond Vet Laboratory Pharma, Argentina). After a 15 cm long skin incision, the subcutaneous tissue was dissected and the TC were inserted and fixed with nonabsorbable suture. The skin was then sutured. TC remained implanted for a 5 week period previous to the pharmacokinetic study, in order to obtain TCF. Cobactan™ preparations used were 4.5% for the IV route and Cobactan™ 2.5% for IM and SC administrations (Intervet International, Heideblick-Beesdau, Brandenburg, Germany). Once the study was completed, tissue cages were removed surgically using the same anesthetic and surgical protocol, without complications.

The study was carried out with a 3×2 cross-over design, in order to so each animal received all treatments after the three experiments, with a washout period of 15 days. IV, IM and SC administrations were performed in the left jugular vein, the thigh and the posterior zone of the elbow on the ribcage, respectively.

2.2. Sampling

Blood samples (3 mL) were collected at 2, 6, 12, 20, 30, 45, 60 and 90 min after IV administration and 5, 10, 20, 30, 45 and 60 min after IM and SC routes, continuing sampling at 2, 3, 4, 6, 8, 10, 12 and 24 h for all routes. 0.5 mL of TCF was extracted aseptically from each tissue cage (total of 1 mL per sample), through perforations present in the device, at 0.5, 1, 3, 6, 9, 12 and 24 h, post administration of CFQ.

Blood and TCF samples were centrifuged and the supernatant was stored at −20 °C until analysis. The time to CFQ determination did not exceed two months.

2.3. Cefquinome analysis in serum and tissue cage fluid

Serum and tissue cage fluid concentrations of CFQ were determined by a modified microbiological assay described by Bennett et al. (1966) using *Klebsiella pneumoniae* ATCC 10031 as test microorganism. Each sample was plated in triplicate and each standard dilution quintupled. The parameters included for the validation of the method were: linearity, accuracy, intra-day and inter-day variation, lower limit of quantification (LLOQ) and lower limit of detection (LLOD) (VICH/GL3, 2008). There was a logarithmic relation between CFQ concentrations and growth inhibition zones, in the range of 0.05–50 μg/mL ($r^2 = 0.96$). For the calculation of accuracy, intra-day and inter-day coefficients of variation, three concentrations were included (0.12, 1 and 50 μg/mL). An accuracy of 100.74, 102.26 and 102.27% were obtained for CFQ concentrations of 0.12, 1 and 50 μg/mL respectively. Intra-day and inter-day coefficients of variation were 8.98 and 9.96%, for 0.12 μg/mL; 6.26 and 6.94% for 1 μg/mL and 1.65 and 2.00% for 50 μg/mL. LLOQ and LLOD were 0.12 μg/mL and 0.05 μg/mL, respectively. The LLOQ was the lower limit of the concentration used in the pharmacokinetic analysis.