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Pharmacokinetics and bioavailability of marbofloxacin in lambs following administration of intravenous, intramuscular and subcutaneous

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ABSTRACT

In this study, the pharmacokinetic disposition and bioavailability of marbofloxacin (MB) were determined in lambs after single intravenous (IV), intramuscular (IM), and subcutaneous (SC) administrations at a dose of 3 mg/kg. The plasma concentration of MB was measured using high-performance liquid chromatography-UV, and the pharmacokinetic parameters were analyzed using a non-compartmental analysis. Following IV, IM, and SC administrations, the mean terminal half-life ($t_{1/2\lambda z}$) was 11.48, 12.64, and 24.86 h, respectively, and the mean residence time (MRT) was 7.27, 7.81, and 10.11 h, respectively. The bioavailability (F) was 96.01 and 126.39%, after IM and SC administration, respectively. This study showed that SC administration of MB at a dose of 3 mg/kg exhibited flip-flop pharmacokinetics in lambs. These results suggested that MB could be useful in the treatment of severe systemic infections, such as those with *M. haemolytica* (MIC = 0.035 µg/mL), in lambs since high $AUC_{0\text{-}24}$/MIC and C_{max}/MIC ratios were achieved after IV and IM administration at 3 mg/kg. However, MB administration (3 mg/kg) *via* the IV, IM, and SC routes might not be effective in the treatment of respiratory infections caused by organisms with MIC_{90} value in lambs.

1. Introduction

Quinolones, which exhibit broad spectrum, rapid bactericidal activity and a low incidence of resistance development, are an important class of antibacterial agents (Foroumadi et al., 2005). Marbofloxacin (MB) [9-fluoro-2,3-dihydro-3 methyl-10 (4-methyl-1-piperazinyl)-7-*oxo*-7H-pyrido (3,2,1-ij) (4,1,2) benzoxadiazine-6 carboxylic acid] is a third-generation fluoroquinolone used and developed only in veterinary medicine (Bryskier and Chantot, 1995). The piperazine and oxadiazine rings in MB are responsible for its different pharmacokinetic properties compared to those of the first generation quinolones, including a larger volume of distribution, longer elimination half-life, and higher bioavailability (Brown, 1996; Fitton, 1992). These pharmacokinetic properties facilitate its distribution throughout the organism and daily applicability. Several studies showed the intracellular accumulation of MB (Gladue et al., 1989), which might facilitate its diffusion into infected tissues, in a manner similar to that suggested for azithromycin.

Respiratory diseases in sheep and lambs represent a significant cause of financial loss for lamb producers (Scott, 2011). According to clinical findings, the major bacteria causing respiratory diseases are *Mannheimia haemolytica* (*M. haemolytica*) and *Mycoplasma* spp. species. In addition, concurrent infections with these microorganisms are common (Bell, 2008; Brogden et al., 1998). MB is recommended for the treatment of respiratory system diseases caused by *M. haemolytica* and *Mycoplasma* species owing to its broad spectrum of bactericidal activity and high efficacy (Aliabadi and Lees, 2002; Skoufos et al., 2007; Thomas et al., 2001). It has been approved for the treatment of various diseases in cattle, pigs, cats, and dogs (Medicines, 1999). Although a dose of 3 mg/kg of MB has not been approved for use in lambs, previous studies have shown that this dosage is clinically effective (Skoufos et al., 2007).

MB has a concentration-dependent characteristic antibacterial activity (Meunier et al., 2004). The minimum inhibitory concentration (MIC) is the most important pharmacodynamic parameter used to determine the effectiveness of the antibacterial drugs against the infectious agents (Craig, 1998). The MIC value together with the pharmacokinetic parameters can provide information about the dosage regimen of the antibacterial drugs (Theuretzbacher, 2012). The most important pharmacokinetic/pharmacodynamic parameters used for the evaluation of antibacterial agents with concentration-dependent bactericidal activity are the area under the concentration-time curve/minimum inhibitor concentration (AUC/MIC) and maximum

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concentrationration/minimum inhibitor concentration (C_{max}/MIC) ratios (Macedo et al., 2013; Müller et al., 2004).

The pharmacokinetic properties of MB have been evaluated in dogs (Bidgood and Papich, 2005), cattle (Shan et al., 2014), calves (Lüders et al., 2012), goats (Waxman et al., 2001), sheep (Sidhu et al., 2010), llamas (Rubio-Langre et al., 2012), horses (Carretero et al., 2002), foals (Tohamy and El-Gendy, 2013), and turkeys (Haritova et al., 2006). However, its pharmacokinetic properties after intravenous (IV), intramuscular (IM) and subcutaneous (SC) administration in lambs are yet to be determined.

In this study, we aimed to (1) investigate the pharmacokinetic disposition of MB in lambs after single IV, IM, and SC administrations at a dose of 3 mg/kg, (2) determine its bioavailability following extravascular administration, and (3) integrate the pharmacokinetic parameters obtained from this study and the MIC values obtained against *M. haemolytica* and *Mycoplasma* species in previous studies. These data could allow for the recommendation of dosage regimens of MB for the treatment of infections caused by susceptible pathogens, such as *M. haemolytica* and *Mycoplasma* species.

2. Material and methods

2.1. Animals

Six clinically healthy Akkaraman male lambs (weight, 15–25 kg; age, 3–5 months) were used in the study. During the experimental period, the lambs were maintained together, outdoors during the day, and housed at night. The animals were housed and fed daily with a rye grass and clover hay mix and a supplementary concentrate. Water was provided ad libitum. The experimental procedures were approved by the Ethics Committee of Dicle University (Diyarbakir, Turkey, Ethics Commission Opinion 28/2016).

2.2. Experimental design

A randomized, three-way crossover (2 × 2 × 2), single-dose study with at least a 2-week washout period was carried out. MB (Marbox®, CEVA, Turkey) was administered to each animal via the IV, IM, and SC routes at a dosage of 3 mg/kg. For IV administrations, MB was injected as a bolus through a catheter placed in the left jugular vein. For IM and SC administration, MB was injected in the semitendinosus muscle and under the lateral rib cage skin, respectively. Blood samples (2 mL) were collected using a catheter placed in the right jugular vein into tubes containing the anticoagulant heparin at 0 (pre-treatment), 5, 10, 15, 20, 25, 30, and 45 min and 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 18, 24, and 36 h post-dosing. Before MB administration, blood samples (10 mL) were obtained from all animals for validation studies. Samples were centrifuged (10 min, 2000 × *g*), and the plasma was stored at −70 °C until assay.

2.3. HPLC assay of MB

Plasma MB concentrations were determined using a modified high-performance liquid chromatography (HPLC) method, as previously described (Potter et al., 2013; Real et al., 2011). Frozen plasma samples (−70 °C) were allowed to reach room temperature. Plasma samples (200 µL) were transferred to micro-centrifuge tubes. After addition of 400 µL of acetonitrile to prevent protein denaturation, the tubes were mixed by vortexing for 30 s and centrifuged at 10,000 rpm for 10 min. The supernatants (100 µL) were transferred into new tubes, 100 µL of water was added, and the tubes were vortexed for 10 s. The supernatants (200 µL) were transferred into auto-sampler vials, and a volume of 10 µL was injected into an HPLC-UV system (Shimadzu, Tokyo, Japan). The HPLC system was controlled by a CBM-20A system, equipped with a low-pressure gradient-flow control valve (LPGE unit) pump (LC-10AT) with a degasser (DGU-14A), auto-sampler (SIL-10AD), and column oven (CTO-10A). Fixation was performed using a SPD-10A UV–vis detector. The column and auto-sampler temperatures were set at 40 °C and room temperature, respectively. MB separation was performed with using a Gemini C18 column (250 × 4.6 mm; internal diameter, 5 µm; Phenomenex, Torrance, CA, USA). UV separation was performed at a wavelength of 280 nm. The mobile phase consisted of 17% acetonitrile (ACN), 0.04% triethylamine, 83% *ortho*-phosphoric acid and was pumped using a low-pressure gradient system. All solutions were filtered using 0.45-µm filters under vacuum before being subjected to liquid chromatography followed by sonication for 20 min. The retention time of the MB was 22.5–23.5 min, with a total run time of 25 min and equilibration time of 2 min. The flow rate was set at 1 mL/min. Data analysis was performed using Asus PC controlled Lc solution software program (Shimadzu, Japan).

Pure MB (CEVA, Turkey) was used for quality control. A working solution of MB was prepared in pure water to obtain a final concentration of 1 mg/mL and stored at −70 °C. The calibration standard curves of blank plasma containing MB were linear between concentrations of 0.04 and 10 µg/mL (r2 > 0.99) MB. The limit of quantitation (LOQ) and limit of detection (LOD) of the method were 0.04 and 0.02 µg/mL, respectively. The interday percent coefficients of variation (CV) were 6.22, 5.37, and 3.55% for MB concentrations of 0.1, 1, and 10 µg/mL, respectively. The intraday percent coefficients of variation (CV) for MB were 4.93, 4.71, and 2.16% for MB concentrations of 0.1, 1, and 10 µg/mL, respectively.

2.4. Pharmacokinetic analysis

Win-Nonlin software (Version 4.1, Pharsight Corporation, Scientific Consulting Inc., North Carolina, USA) was used to determine the pharmacokinetic parameters of MB. The pharmacokinetic variables for each animal were determined using non-compartmental model analysis. The maximum observed plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were obtained directly from the concentration–time data. Area under the plasma concentration–time curve from time 0 to the time of the last measurable concentration (AUC) was computed using mixed log-linear trapezoidal rule. The AUC from time 0 to infinity ($AUC_{0-\infty}$) after a single dose was computed as the sum of AUC_{0-36} and $C_{last/\lambda z}$. Terminal elimination ($t_{1/2\lambda z}$) was calculated as $\ln(2)/\lambda_z$. The bioavailability (F) was calculated with using the AUC values following SC, IM and IV administration ($F = AUC_{SC,IM} \times 100/AUC_{IV}$).

2.5. Pharmacokinetic-pharmacodynamic indices

The pharmacokinetic-pharmacodynamic indices (AUC_{0-24}/MIC and C_{max}/MIC) of MB against *M. haemolytica* and *Mycoplasma* species after the IV, IM, and SC administrations were integrated using the pharmacokinetic parameters obtained in this study and the MIC values obtained in previous studies against *M. haemolytica* (Kroemer et al., 2012; Sidhu et al., 2010) and *Mycoplasma mycoides subsp. capri* strains (Paterna et al., 2016), with an MIC value of 0.035 µg/mL and an MIC value of 0.1 µg/mL, respectively.

2.6. Statistical analysis

Statistical analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago, IL). A p-value < 0.05 was considered statistically significant. The harmonic mean ± standart deviation (SD) was estimated for the time parameters [mean residence time (MRT) and elimination half-life ($t_{1/2\lambda z}$)], and statistical significance was evaluated using Wilcoxon rank-sum test. The T_{max} was presented as the median and tested by Wilcoxon rank-sum test for significant differences. Comparisons of the C_{max} and F were performed using a paired *t*-test. Differences in other pharmacokinetic parameters (mean ± SD) were evaluated using one-way analysis of variance (ANOVA) test, whereas and the statistical differences between different administration routes