Pharmacokinetics of marbofloxacin, after single intravenous administrations, in buffaloes calves

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ABSTRACT: Marbofloxacin is a synthetic, bactericidal antimicrobial, belonging to the fluoroquinolone group which acts by inhibition of DNA gyrase and those acts by concentration dependant killing mechanism, so high plasma concentration initially is important. This drug is a fluoroquinolone developed exclusively for veterinary use, and exhibit high bactericidal activity against a broad spectrum of aerobic gram-negative, some gram-positive bacteria and Mycoplasma spp. The pharmacokinetic behaviour of marbofloxacin was investigated after intravenous (2 mg/kg) in five clinically healthy buffaloes (10 days-old). Plasma concentrations of the marbofloxacin were determined by a HPLC/u.v. method. After intravenous administration, marbofloxacin in buffaloes was characterized by a AUC = 8.42±3.71 μg·h/ml, a large volume of distribution (Vss=1.59±0.55 L/kg) and a long persistence with an elimination half-life (t½) of 4.6±0.31 h, and MRT 5.90±0.57h. Furthermore, marbofloxacin in buffaloes was characterized by a relatively low total body clearance (Cl) of 0.28±0.12 L/kg·h.

Key words: Fluoroquinolone, Marbofloxacin, Buffalo, Farmacokinetic.

INTRODUCTION - Marbofloxacin is a fluoroquinolone antimicrobial agent, developed exclusively for veterinary use, which acts by inhibition of DNA gyrase and those act by concentration dependant killing mechanism, so high plasma concentration initially is important. Marbofloxacin has an extended spectrum of bactericidal activity which includes mainly Gram-negative pathogens and some Gram-positive pathogens such as Staphylococcus spp., Mycoplasma spp. and Pseudomonas spp and Mycoplasma spp (Meunier et al., 2004). There are numerous potential indications for fluoroquinolone treatment in ruminant medicine.

In the past, the therapeutic recommendations applied to a single ruminant species were extrapolated to the others because no important differences among cattle, sheep, goats and buffaloes were recognized. However, a different pharmacokinetic behavior of antimicrobials has been described along the ruminant species (Elsheikh, 1997). It is important to know the pharmacokinetics of the drugs in each species, in order to minimize dosage errors, which
could lead to therapeutic failures, toxic effects or bacterial resistance development. Physiological differences between buffaloes and others ruminant (such as corporal composition, percentage of adipose tissue, reproductive cycle, hepatic metabolism or renal excretion) have been described (Groves, 1989). These factors could influence the disposition of drugs and, therefore, these species could require different dosage.

**MATERIAL AND METHODS** - The experiment was performed in five male buffaloes 10 days old and weighing 48 ± 4 kg. A complete clinical and haematological evaluation was performed throughout the study. The study was approved by Institutional Animal Use Committee.

**Experimental design**
All animals first received a 2 mg/kg b.w. dose of 10% aqueous solution of marbofloxacin (Marbocyl®, Vétoquinol, Lure, France) intravenously into the right jugular vein. Blood samples were collected through a catheter placed in the left jugular vein, at 0, 5, 10, 15, 30, 45 min, and 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 24, h after administration. The samples were centrifuged at 1800 g for 20 min within 30 min after collection. Plasma aliquots were frozen (-80°C) until assayed.

**Analytical assay**
The plasma marbofloxacin concentrations were determined by high performance liquid chromatography (HPLC) modified from a previously published method (Schneider et al., 1996). Marbofloxacin was provided by Vétoquinol, Lure, France and ofloxacin was used as internal standard (Sigma Chemical CO., St. Louis, MO, USA). The UV detection wavelength was 295 nm and the flow rate was 1 ml/min. The quantification limit was 0.025 μg/ml and the method was linear between 0.025 μg/ml and 15 μg/ml. The inter-assay and intra-assay reproducibility were below 10% (coefficient of variation).

**Sampling process**
A volume of 300 μl of plasma was placed into 15 ml screw-capped tubes. 75 microliters 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 during 10 min in a horizontal agitator, the samples were centrifuged at 3200 g for 7 min and the organic layer transferred to another tube from where it was evaporated under nitrogen stream at 40 °C. The samples were redissolved in 150 μl of mobile phase and a 50 μl aliquot was injected into the HPLC system.

**Pharmacokinetic analysis**
Plasma levels of marbofloxacin were subjected to model independent analysis, statistical moments were used to compute the non compartmental with the help of PCnonlin® V 4.0 software package. The terminal half-life (t_{1/2λ}) was calculated by t_{1/2λ} = 0.693/β. Total plasma clearance (Cl) was calculated by means of the equation Cl = Dose i.v./AUC and the volume of distribution at steady-state (V_{ss}) was determined as follows: V_{ss} = Dose i.v. MRT, where MRT is the mean residence time, model of area under the concentration-time curves (AUC_{∞}) and mean residence time (MRT).

**RESULTS AND CONCLUSIONS** - After intravenous administration, marbofloxacin in buffaloes was characterized by a large volume of distribution (Vss=1.59±0.55 L/kg). This result is similar than that described by other ruminant species (Waxman et al., 2001; Schneider et al., 2004; Shojaee Aliabadi and Lees, 2002). Also, a relatively low total body clearance (Cl) of 0.28±0.12 L/kg·h was obtained, similar than those described in goats (Cl=0.23 mL/kg·h, Waxman et al., 2001), in horse (0.19 mL/kg·h Carretero et al., 2002; 0.25 mL/kg·h
Bousquet Melou et al., 2002) and sheep (0.25 mL/kg h Shem-Tov et al., 1997), in calf (0.21 mL/kg h; Shojaee and Lees, 2002). An AUC of 8.42±3.71 μg·h/ml was observed in buffaloes. The influence of this clearance produce a relatively long persistence with an elimination half-life (t½) of 4.6±0.31 h, and MRT 5.90±0.57h. It is in accordance with the pharmacokinetic behaviour observed in other herbivorous species as horses (Carretero et al., 2002) or calf (Shojaee and Lees, 2002); however, it is lower than those related to donkey (9.24 h; Gonzalez et al., 2007) or goats (Waxman et al., 2001).

Figure 1. Marbofloxacin plasma concentration vs time curve obtained after intravenous administration at the dosage of 2 mg/kg in buffaloes.

Table 1. Pharmacokinetic parameters obtained after intravenous administration of marbofloxacin (2 mg/Kg) to buffaloes.

| Pharmacokinetic parameter | mean | SD  |
|---------------------------|------|-----|
| λ (h⁻¹)                   | 0.1513 | 0.0107 |
| T₁/₂λ (h)                 | 4.60 | 0.31 |
| AUC (μg·h/mL)             | 8.65 | 3.86 |
| MRT (h)                   | 6.51 | 0.68 |
| CI (L/kg·h)               | 0.28 | 0.12 |
| Vss (L/kg)                | 1.59 | 0.55 |

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