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Pharmacokinetics and bioavailability of moxifloxacin in buffalo calves

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ABSTRACT

The pharmacokinetics of moxifloxacin were investigated in buffalo calves following a single intravenous and intramuscular administration of moxifloxacin (5 mg kg^{-1} body wt.). Moxifloxacin concentrations in plasma and urine were determined by microbiological assay. Pharmacokinetic analysis of disposition data indicated that intravenous administration data were best described by a two compartment open model, whereas intramuscular administration data were best described by a one compartment open model. Following intravenous administration, the elimination half life ($t_{1/2\beta}$), volume of distribution ($Vd_{(area)}$) and total body clearance were 2.69 ± 0.14 h, 1.43 ± 0.08 L kg^{-1} and 371.2 ± 11.2 ml kg^{-1} h^{-1}, respectively. Following intramuscular administration, the absorption half life ($t_{1/2ka}$) was 0.83 ± 0.20 h. The systemic bioavailability (F) of moxifloxacin in buffalo calves was 80.0 ± 4.08%. Urinary excretion of moxifloxacin was less than 14% after 24 h of administration of drug. *In vitro* binding of moxifloxacin to plasma proteins of buffalo calves was 28.4 ± 3.77%. From the data of surrogate markers (AUC/MIC, C_{max}/MIC), it was determined in the buffalo calves that when administered by intravenous or intramuscular route at 5 mg kg^{-1}, moxifloxacin is likely to be effective against bacterial isolates with MIC $\leqslant$ 0.1 μg ml^{-1}.

1. Introduction

Moxifloxacin is a novel fourth generation fluoroquinolone with a broad spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria, anaerobes and atypical organisms such as Mycoplasma and Chlamydia species. It has the highest potency in its class against *Staphylococcus aureus* and *Staphylococcus epidermidis* (Kowalski et al., 2003). In other species, the lower MIC value and high serum and tissue concentrations make this a suitable antibiotic for treating various infectious diseases including those of upper and lower respiratory tract (Blondeau and Hansen, 2001). Pharmacokinetic studies of antimicrobial agents, which provide a basis for the determination of satisfactory dosage regimen, are relevant when they are undertaken in the species in which the drugs are to be used clinically. There have been some limited data published on the pharmacokinetics of moxifloxacin in animals. Pharmacokinetic properties of moxifloxacin have been reported in horses (Gardner et al., 2004), rabbits (Fernandez-Varon et al., 2005; Carceles et al., 2006), lactating goats (Fernandez-Varon et al., 2006; Carceles et al., 2007), ewes (Goudah, 2008), camels (Abd El-Aty et al., 2007) and humans (Sullivan et al., 1999; Siefert et al., 1999b). As the usefulness of an antibacterial agent depends on its efficacy, safety and pharmacokinetic disposition in the target animal, the aim of present study was to investigate the pharmacokinetics of moxifloxacin following single intravenous and intramuscular administration in buffalo calves. Specifically the present study was planned to determine the pharmacokinetics, bioavailability, urinary excretion and *in vitro* plasma protein binding of moxifloxacin in buffalo calves. The results of this investigation will facilitate the judicious and efficacious use of moxifloxacin in buffalo calves due to its high plasma and tissue concentrations.

2. Methods

2.1. Animals

Six intact healthy male buffalo calves ranging between 6–12 months of age and weighing between 80 and 150 kg body weight were used. The animals were housed in an animal shed with concrete floor and adequate ventilation. The animals were determined to be clinically healthy before the study. All the animals were acclimatized in the animal shed under uniform conditions and were maintained on green fodder, wheat straw and water *ad libitum*. They did not receive any drug treatment before the study. For the collection of urine, the experimental animals were kept in metabolic stalls of standard size, 12 h before the start of experiment and kept there for entire study. The metabolic stalls are designed in such a way that urine voided by animals can be collected at any time interval without any spillage. Before the start of experiment, permission for experiments on these animals was obtained from the Institutional Animal Ethics Committee (IAEC).

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2.2. Experimental design

The study was performed in two phases, using a cross over design with a washout period of 21 days. Aqueous solutions of a commercially available moxifloxacin hydrochloride were administered by either the intravenous or the intramuscular route at single doses of 5 mg kg^{-1} body weight. Animals were randomly assigned to receive intravenous or intramuscular doses first. Intravenous injection of moxifloxacin was into the jugular vein and intramuscular administration was performed in the lower third region of the neck muscles. On each occasion blood samples (4–6 ml each) were withdrawn by venipuncture from the contra lateral jugular vein into heparinized glass test tubes before administration and at 1, 2.5, 5.0, 10, 15, 30, 45, 60 min and 2, 3, 4, 5, 6,7, 8, 9, 10, 12, 16 and 24 h after administration of the drug. Plasma was collected after centrifugation at 2000*g* for 15 min at room temperature and kept at −20 °C until analysis usually the next day. Urine was collected at 4, 8, 12, 20 and 24 h after administration of drug. The urine voided by animals was filtered and measured and approximately 5 ml urine sample was stored at −20 °C till analysis. The moxifloxacin is stable in human plasma when stored at −20 °C for 2 months and room temperature for 24 h (Tatar Ulu, 2007). However stability of moxifloxacin in stored samples of buffalo calves was not tested.

2.3. Bioassay

The concentrations of moxifloxacin in plasma and urine were estimated by employing the microbiological assay technique (Simon and Jongyin, 1970; Arret et al., 1971) using *Escherichia coli* (MTCC 739) as the test organism. Three alternate wells on assay plates were filled with reference concentration (0.5 μg ml^{-1}) and remaining three with a known concentration of drug. Three plates were used for each concentration. These assay plates were incubated at 37 °C for a period of 24 h. At the end of incubation, the diameter of zones of inhibition were measured and the moxifloxacin concentrations in samples were calculated from the standard curve and expressed as μg ml^{-1}. The bioassay method used in this work could not distinguish between the parent compound and its active metabolites, if they exist. However, it measured the overall microbiological activity of the drug. The standard curve of moxifloxacin in buffalo calf plasma was linear between 0.125 and 1 μg ml^{-1}. The value of coefficient of determination (r^2) of the standard curve was 0.99. The drug could be detected up to a minimum limit of 0.1 μg ml^{-1}. The moxifloxacin recovery exceeded 93% from plasma and urine over the concentration range of 0.125–15 μg ml^{-1}. The intra-day and inter-day coefficients of variance were less than 5% and 10%, respectively.

2.4. Pharmacokinetic analysis

The plasma concentration time data for each buffalo calf were determined according to the computed least squares regression technique. The pharmacokinetic analysis of moxifloxacin in plasma was performed by using WinNonlin® Version 5.2 (Pharsight Corporation). Lowest weighted sum of squares and lowest Akaike's information criterion value for the individual data was applied to select the model (Yamaoka et al., 1978). Plasma moxifloxacin concentration versus time decreased in a bi-exponential manner following intravenous injection for which polyexponential equation is:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

where C_p is the concentration of drug in the plasma at time t; A is the intercept of the distribution phase with the concentration axis expressed as μg ml^{-1}; B is the intercept of the elimination phase with the concentration axis expressed as μg ml^{-1}; α is the distribution rate constant expressed in units of reciprocal time (per hour); β is the elimination rate constant expressed in units of reciprocal time (per hour), and e is the natural logarithm base.

The kinetic parameters were calculated from the formulae derived for a single and two compartment open model (Notari, 1980; Gibaldi and Perrier, 1982).

2.5. In vitro plasma protein binding

In vitro binding of moxifloxacin to plasma protein was determined by employing the equilibrium dialysis technique (Kunin et al., 1959). The dialyzing bags (4 Å pore size) 10 cm long were washed with distilled water and soaked overnight in phosphate buffer (0.2 M, pH 7.4). The various concentrations of moxifloxacin e.g. 6.25, 12.5, 25, 50, 100 μg ml^{-1} were prepared in plasma taken from untreated animals. Each dialyzing bag was knotted on one end before filling 5 ml of plasma containing known amount of drug and the other end was then securely tied. Each bag was immersed in separate tubes containing 5 ml of phosphate buffer and the tubes were incubated at 37 °C for 24 h with occasional shaking. At the end of incubation period phosphate buffer as well as contents of the dialyzing bags was separately analyzed for the concentration of moxifloxacin. Dialysis was performed in triplicates for each concentration. The extent of *in vitro* plasma protein binding of moxifloxacin was calculated by the following equation.

$$\text{Percent of moxifloxacin bound to plasma protein} = \frac{C'_p - C_W}{C_p} \times 100$$

where,

C'_p = concentration of moxifloxacin in plasma after incubation.
C_W = concentration of moxifloxacin in phosphate buffer after incubation.
C_p = concentration of moxifloxacin in plasma before incubation.

3. Results

The mean (±SE) plasma concentration of moxifloxacin following intravenous and intramuscular administration are shown in Fig. 1 and Table 1. Mean (±SE) values for pharmacokinetic parameters are given in Table 2. The moxifloxacin plasma concentration versus time data after intravenous administration could best be described by a two compartment open model. The distribution and elimina-

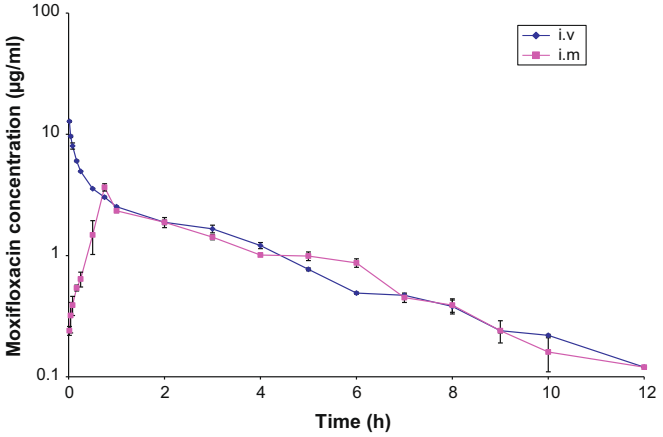

Fig. 1. A semilogarithmic plot of plasma levels (mean ± SE) versus time of moxifloxacin following intravenous and intramuscular administration (5 mg kg^{-1} body weight) in buffalo calves (n = 6).