Pharmacokinetics of pazufloxacin in buffalo calves after single subcutaneous administration

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ABSTRACT

The pharmacokinetics and urinary excretion of pazufloxacin was investigated in buffalo calves following a single subcutaneous administration (5 mg/kg b.wt.). Pharmacokinetic analysis of disposition data indicated that subcutaneous administration data were best described by 1-compartment open model. The peak plasma levels of pazufloxacin were 1.12±0.05 μg/ml at 45 min and the drug was detected up to 16 h. The absorption half-life and elimination half-life were 0.26±0.04 h and 3.01±0.18h, respectively. The apparent volume of distribution and total body clearance were 2.96±0.20 L/kg and 0.67±0.02 L/kg/h, respectively. The urinary excretion of pazufloxacin in 24 h was 23.8±2.30 % of total administrated dose. From the data of surrogate markers (AUC/MIC, Cmax/MIC), it was determined in the buffalo calves that when administered by the subcutaneous route at 5 mg/kg, pazufloxacin is likely to be effective against bacterial isolates with MIC ≤ 0.05 μg/ml.

Key words: Buffalo calves, Pazufloxacin, Pharmacokinetics, Urinary excretion

The presence of aminoacyl group at C-10 is a unique feature of pazufloxacin molecule imparting potent broad spectrum activity against gram-positive and gram-negative bacteria including variety of resistant strains and anaerobic bacteria (Zhanel et al. 2002, 2006). The antibacterial activities of pazufloxacin are superior to those of ceftazidime, ceftriaxone, imipenem/cilastatin, meropenem against methicillin resistant Streptococcus aureus, ampicillin-resistant Haemophilus influenzae, ESBL possessing Klebsiella pneumonia, and imipenem/cilastatin resistant Pseudomonas aeruginosa (Mitsuyama et al. 1999). Pharmacokinetic properties of pazufloxacin were reported in febrile buffalo calves (Sharma et al. 2014), rats (Nakata et al. 1999, Lou et al. 2007), and mice and rabbits (Nakata et al. 1999). The purpose of this study was to determine the pharmacokinetics, urinary excretion and appropriate dosage regimen of pazufloxacin in buffalo calves after a single subcutaneous administration.

MATERIALS AND METHODS

Healthy male buffalo calves (6), weighing 80–150 kg, and 6–12 months of age were kept in the departmental animal shed with concrete floor and adequate ventilation. A constant supply of water was maintained in the shed. All the animals were acclimatized in the animal shed under uniform conditions and were maintained on green fodder and wheat straw and water ad lib. On the day of experiment, the animals were kept in standard metabolic stalls, designed so that all the urine passed by the animals over a particular period could be collected without any contamination or spillage. Pazufloxacin was given by subcutaneous route @ 5 mg/kg body weight. Blood samples (4–5 ml each) were withdrawn from the jugular vein into heparinized glass test tubes before administration and at 2.5, 5, 10, 15, 30, 45 and 60 min and 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 16 h after administration of the drug. Plasma samples (500 μl) were vortex mixed for 10 s with 100 μl internal standard solution (10 μg/ml). After addition of 400 μl acetonitrile, the mixture was vortex mixed for 5 min, left at room temperature for 30 min, and centrifuged at 2,000 g for 15 min at room temperature and then stored at –20°C until analysis, usually the next day. The urine samples were collected at 4, 8, 12, 20 and 24 h after drug administration. The plasma standards/samples (5 μl, 4.6 × 250 nm) was used as a stationary phase. The mobile phase consists of buffer (20 mM citric acid and 5 mM of 1-octane sulfonic acid, pH 5 was adjusted with sodium hydroxide) and acetonitrile was mixed in the ratio of 81:19 v/v. The flow rate of mobile phase was 1 ml/min. The detector for plasma sample was performed with fluorescent detector at 330 nm (excitation) and 394 nm (emission). Samples were analyzed for 28 min. Plasma standards/samples (500 μl) were vortex mixed for 10 s with 100 μl internal standard solution (10 μg/ml). After addition of 400 μl acetonitrile, the mixture was vortex mixed for 5 min, left at room temperature for 30 min, and centrifuged at 2,000 g for 15 min at room temperature and then stored at –20°C until analysis, usually the next day. The urine samples were collected at 4, 8, 12, 20 and 24 h after drug administration. The volume of urine was measured and approximately 8–10 ml was frozen for drug analysis.

Plasma concentrations of pazufloxacin were determined using high performance liquid chromatography (Phapale et al. 2010). A reverse phase c18 column (particle size 5 μ, 4.6 × 250 mm) was used as a stationary phase. The mobile phase consists of buffer (20 mM citric acid and 5 mM of 1-octane sulfonic acid, pH 5 was adjusted with sodium hydroxide) and acetonitrile was mixed in the ratio of 81:19 v/v. The flow rate of mobile phase was 1 ml/min. The detector for plasma sample was performed with fluorescent detector at 330 nm (excitation) and 394 nm (emission). Samples were analyzed for 28 min. Plasma standards/samples (500 μl) were vortex mixed for 10 s with 100 μl internal standard solution (10 μg/ml). After addition of 400 μl acetonitrile, the mixture was vortex mixed for 5 min, left at room temperature for 30 min, and centrifuged at 2,000 g for 15 min at room temperature and then stored at –20°C until analysis, usually the next day. The urine samples were collected at 4, 8, 12, 20 and 24 h after drug administration. The volume of urine was measured and approximately 8–10 ml was frozen for drug analysis.

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10,000 rpm for 10 min. The supernatant (200 μl) was then vortex mixed with 800 μl buffer for 5 min, and 1 μl of the solution obtained was injected for LC analysis. Buffer used in the mobile phase and for sample preparation was prepared by mixing aqueous 20 mM citric acid (2.1 g) and 5mM 1-octane sulfonic acid (0.55 g) salt in 750 ml of HPLC water. Volume of the buffer was raised to 800 ml with HPLC water. An 81:19 (v/v) mixture of buffer and acetonitrile was used as isocratic mobile phase. The mobile phase was delivered at a flow rate of 1.0 ml/min. Before use, the mobile phase was filtered through a 0.22 μm millipore filter.

The calibration curve for plasma samples was constructed in the range of 0.02–2.5 μg/ml. The limit of detection was 0.02 μg/ml in plasma. The value of regression coefficient (γ) was 0.99. Mean recovery from plasma at concentration 0.02 to 2 μg/ml ranged from 89–100 %. For plasma sample overall RSD was less than 10%. The kinetic parameters were calculated from the formulae derived for a mono compartment open model (Gibaldi and Perrier 1982). The dosage regimen (D) of pazufloxacin was also determined based on kinetic data (Baggot 1977) by using following formulæ:

\[ D = C_p^{(min)} \cdot V_d(e^h) \]

where \(C_p^{(min)}\) is the minimum therapeutic concentration of pazufloxacin, \(t\) is the dosage interval and other parameters are defined in Table 1.

**RESULTS AND DISCUSSION**

The mean (±SE) plasma concentration of pazufloxacin following subcutaneous administration are shown in Fig. 1. Evaluation of the results on observed plasma levels of pazufloxacin indicated that the data can be best fitted to one-compartment open model with the exponential equation \(C_p = B e^{-t} \cdot A e^{-kt}\), where \(C_p\) is the pazufloxacin concentration at time \(t\), \(A\) and \(B\) are zero-time intercepts of absorption and elimination phases of the plasma concentration-time curves, respectively; \(K_a\) and \(\beta\) are the absorption and elimination rate constants, respectively, and \(e\) represents the base of natural logarithms. The minimum therapeutic plasma concentration (0.05 μg/ml) was maintained from 2.5 to 12 h. The plasma concentration vs time curve after subcutaneous administration documented mean \(C_{max}\) at 0.83 h, indicating fast absorption. The rapid appearance of pazufloxacin in the plasma suggests that this drug quickly enters into the systemic circulation following subcutaneous administration. Mean (±SE) values for pharmacokinetic parameters are given in Table 1. The elimination half life (3.01±0.18 h) obtained in our study, does not offer advantages to pazufloxacin over other fluoroquinolones. The value of elimination half life (\(t_{1/2β}\)) of buffalo calves is longer than the corresponding values reported in rabbits, rats and mice, but it is shorter than the values reported in dogs (Nakata et al. 1999), \(t_{1/2β}\) in rabbits, rats, mice and dogs was reported at 1.0, 0.88, 0.23, and 4.5 h, respectively.

Pazufloxacin exhibited a relatively high volume of distribution (2.96±0.20 L/kg) in buffalo calves showing that there is relatively quick and wide distribution of pazufloxacin after subcutaneous administration. The volume of distribution suggested a wide penetration through biological membrane and good tissue distribution. The total body clearance (\(Cl_d = 0.67±0.02 L/kg/h\)) suggested medium elimination of pazufloxacin in buffalo calves. The \(Cl_d\) value of danofloxacin (Sappal et al. 2009), moxifloxacin (Pathania et al. 2007), gatifloxacin (Raipuria et al. 2007) in buffalo calves were 0.71±0.10, 0.37±0.11 and 0.24±0.08 respectively.

**Table 1. Pharmacokinetic parameters of pazufloxacin in buffalo calves (n=6) after a single subcutaneous injection (5 mg/kg body weight)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>μg/ml</td>
<td>1.16±0.01</td>
</tr>
<tr>
<td>(K_a)</td>
<td>h</td>
<td>2.83±0.38</td>
</tr>
<tr>
<td>(t_{1/2Ka})</td>
<td>h</td>
<td>0.26±0.04</td>
</tr>
<tr>
<td>(B)</td>
<td>μg/ml</td>
<td>1.37±0.11</td>
</tr>
<tr>
<td>(t_{1/2β})</td>
<td>h</td>
<td>0.23±0.01</td>
</tr>
<tr>
<td>(V_d(area))</td>
<td>L/kg</td>
<td>2.96±0.20</td>
</tr>
<tr>
<td>(V_d(B))</td>
<td>L/kg</td>
<td>3.81±0.39</td>
</tr>
<tr>
<td>(Cl_d)</td>
<td>L/kg/h</td>
<td>0.67±0.02</td>
</tr>
<tr>
<td>(C_{max})</td>
<td>μg/ml</td>
<td>1.17±0.04</td>
</tr>
<tr>
<td>(t_{max})</td>
<td>h</td>
<td>0.83±0.05</td>
</tr>
</tbody>
</table>

The kinetics parameters are as described by Gibaldi and Perrier (1982). A and B, zero-time plasma drug concentration intercept of the regression line of absorption and elimination phases, respectively; \(K_a\) and \(β\) are the absorption and elimination rate constants, respectively; \(t_{1/2Ka}\) absorption half-life; \(t_{1/2β}\) elimination half-life; \(AUC\), area under the plasma concentration-time curve; \(AUMC\), area under the first-moment curve; \(V_d(area)\), apparent volume of distribution based on \(AUC\); \(V_d(B)\), volume of distribution based on zero-time plasma drug concentration intercept of elimination phase; \(Cl_d\), total body clearance; \(C_{max}\), the peak or maximum plasma concentration; \(t_{max}\), the time to reach peak or maximum plasma concentration.
A concentration of 269.8±42.0 μg/ml in urine up to 24 h of administration. Approximately 24% of total administered drug was recovered in urine after 24 h. Urinary excretion rates of pazufloxacin as the active form within 24 h after administration were 44.7% of the dose in mice, 74.3% in rats, 54.9% in rabbits, and 56.6% in dogs. The concentration of pazufloxacin in urine of buffalo calves remained higher than the MIC (0.0125 - 12.5 μg/ml) of most microorganisms (Nomura et al. 2002) sensitive to the drug up to 24 h. This suggested that use of pazufloxacin in buffalo calves might achieve successful bacterial killing in urinary tract infections. However, we have not performed studies correlating urine concentration and MICs with efficacy. Incomplete and variant urinary pazufloxacin recovery may be explained by several hypotheses: (i) 24-h urine collection was inadequate to collect 100% of the excreted dose, (ii) degradation of pazufloxacin in urine and blood may occur either in vivo or in vitro, (iii) pazufloxacin may be metabolized, or (iv) the drug may be excreted through an alternate pathway such as bile. Since fecal pazufloxacin concentrations were not measured, it is impossible to rule out biliary excretion as an elimination pathway.

The main objective of present study was to determine a satisfactory dosage regimen of pazufloxacin in buffalo calves. It is not axiomatic to compute the dosage regimen of pazufloxacin to be used effectively in clinical practice for the treatment of mild to severe bacterial infections, without having first conducted a detailed pharmacokinetic study. Taking 8 and 12 h as convenient dosage interval (t) and using the values of β and Vd(area) of Table 1, the dosage regimens for pazufloxacin were computed in buffalo calves and presented in Table 3.
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REFERENCES


