DISPOSITION KINETICS AND URINARY EXCRETION OF OFLOXACIN FOLLOWING INTRAMUSCULAR ADMINISTRATION IN BUFFALO CALVES

Sandeep Kumar, J. S. Punia and S. K. Jain

ABSTRACT

Pharmacokinetics and urinary excretion of ofloxacin were determined in male buffalo calves following single intramuscular administration (5 mg.kg⁻¹). The disposition of ofloxacin followed a one-compartment open model after the absorption phase following a single i.m. injection. The drug was rapidly absorbed and at 5 minute, the concentration was 2.55 μg.ml⁻¹. The peak plasma level was 3.78 μg.ml⁻¹ at 30 minute; this declined gradually to 0.54 μg.ml⁻¹ at 360 minute. The drug remained above the minimum therapeutic concentration (MIC₉₀: 0.5 μg.ml⁻¹) for 6 h. The absorption rate constant (ka) was 3.70 h⁻¹ with an absorption half life (t₁/₂ka) of 0.19 h. The observed peak plasma concentration (Cmax) was 3.78 μg.ml⁻¹ at 30 minute (tₘₐₓ). The drug was not detected in plasma after 6 h, as also indicated by a high value of the elimination rate constant (β: 0.367 h⁻¹) and a short biological half life (t₁/₂β: 1.91 h). The volume of distribution (Vz(area): 0.69 L.kg⁻¹) revealed moderate to good distribution and tissue penetration of ofloxacin. The total body clearance (ClB) was 0.25 L.kg⁻¹.h⁻¹. The area under plasma concentration curve (AUC) was 12.40 μg.ml⁻¹.h. About 54 percent of the total administered dose of ofloxacin was excreted in urine in 48 h. An optimal dosage regimen of ofloxacin following a single intramuscular injection in buffalo calves would be 6.5 mg.kg⁻¹ followed by 6 mg.kg⁻¹ at 8 h intervals to maintain a minimum therapeutic concentration of 0.5 μg.ml⁻¹ suitable for the treatment of systemic infections in buffalo calves.

Keywords: buffalo calves, ofloxacin, pharmacokinetics, urinary excretion

INTRODUCTION

Fluoroquinolones are broad spectrum antimicrobial compounds that have very few side effects and do not develop microbial resistance rapidly (Andriole, 1993; Hooper, 2000). Large volume of distribution, better tissue penetration, good bioavailability, and long biological half-lives have made them suitable alternative agents against various infectious diseases. Ofloxacin is a new fluoroquinolone compound having wide therapeutic usefulness in the treatment of bacterial infections in human and veterinary medicine (Blomer et al., 1986; Davies et al., 1986). It is effective against gram-positive and gram-negative pathogens, anaerobes (Varoli and Ferri, 1987), including mycoplasma, rickettsia as well as against multidrug-resistant microorganisms (Brown, 1996; McKeller, 1996). Pharmacokinetics of ofloxacin have been studied extensively in human beings (Fillastre et al., 1987; Lode et al., 1987; Bitar et al., 1989; Orlando et al., 1992). A few reports on pharmacokinetic profiles of ofloxacin are available in various animal species, viz., rabbit (Marangos et al., 1997), dog (Yoshida et al., 1998), pig (Son et al., 2000), goat (Maulik and Ghose, 2000), calves (Gaur et al., 2002; Mohan and Garg, 2002), neonatal calves (Gaur et al., 2004) and chickens (Liu and Fung, 1997; Kalaiselvi et al., 2006). However, data on the pharmacokinetics of ofloxacin in large ruminants especially buffaloes are lacking. The extrapolation of kinetic data from one animal species to other should be avoided as it may result in failure of therapy or even toxicity.

The present investigation was, therefore, undertaken to study the levels of ofloxacin in plasma and urine at different time intervals following its
intramuscular administration, determination of various pharmacokinetic profiles, computation of dosage regimen and urinary excretion of ofloxacin in male buffalo calves (*Bubalus bubalis*) for its rational and judicious therapeutic use in buffalo species.

**MATERIALS AND METHODS**

Six healthy male buffalo calves of Murrah breed weighing 130-180 kg were acclimatized for 2 weeks in the well-ventilated and lighted animal shed of the Buffalo Research Centre of the University prior to commencement of the experiment. The animals were maintained under standard management conditions and provided standard ration (seasonal green fodder, wheat straw and concentrate mixture). The animals had access to feed and water ad libitum.

An injectable preparation of ofloxacin (OLONE™) was procured from Rodec Pharmaceutical Pvt. Ltd., New Delhi, India and injected at the dose rate of 5 mg.kg⁻¹ by the intramuscular route.

**Collection of blood and urine samples and assay**

Blood samples were collected at various time intervals from the opposite contra-lateral jugular venipuncture in heparinized tubes following intramuscular administration of ofloxacin 5 mg.kg⁻¹. Samples were collected prior to and at various times from 5 to 540 minutes. Plasma was separated in disposable cryo-vials and stored at -20°C until assayed.

Urine was collected at regular time intervals using specially designed urine collection bags. The total volumes of urine voided during 0-3, 3-6, 6-9, 9-12, 12-24, 24-36 and 36-48 h intervals were collected. An aliquot of urine was then taken and centrifuged to settle the suspended particles. The supernatant layer of urine free from visible impurities was then filtered through Whatman filter paper No. 1. The filtrate was taken in disposable cryo-vials and stored at -20°C and processed for analysis within 48 h of collection.

Ofloxacin in plasma and urine was determined spectrophotometrically by modifying the method as described for norfloxacin by Jha *et al.* (1996). Ultraviolet absorbance was measured at 295.88 nm for plasma and 352 nm for urine. The limit of detection was 0.2 μg/ml in plasma and 0.7 μg/ml in urine.

**Analysis of data**

Plasma concentration versus time data of ofloxacin of each animal was analysed to determine the various pharmacokinetic parameters of ofloxacin (Gibaldi and Perrier, 1982). Based on pharmacokinetic data, a dosage regimen of ofloxacin was computed (Notari, 1980). Statistical calculations were done as per standard methods and the results are presented as mean ± SE.

**RESULTS AND DISCUSSION**

The drug was rapidly absorbed and detected in plasma within 5 minute of i.m. injection (Table 1). The peak plasma level was 3.78 ± 0.07 μg.ml⁻¹ at 30 minutes. Thereafter, the plasma levels declined gradually. The plasma concentration of ofloxacin was 0.54 ± 0.04 μg.ml⁻¹ at 6 h and thereafter, could not be detected in plasma, which is in agreement with the findings in calves (Mohan and Garg, 2002). However, ofloxacin has been detected up to 48 h in plasma of neonatal calves (Gaur *et al.*, 2002). A lower peak plasma concentration of 1.85 μg.ml⁻¹ at 30 minutes has been reported following i.m. administration of ofloxacin in calves with t₁/₂ka value of 0.17 h (Mohan and Garg, 2002). But in neonatal calves, a peak plasma concentration of 1.33 μg.ml⁻¹ at 60 minutes with a t₁/₂ka value of 0.39 h has been observed (Gaur *et al.*, 2002).

Plasma concentrations versus time data of ofloxacin following single i.m. administration in buffalo calves were analysed using a one-compartment open model, as the semi-logarithmic plot of plasma ofloxacin concentration versus time data revealed a mono-phasic decline in plasma drug concentration. The disposition of ofloxacin has also been reported to follow a one compartment open model with first order absorption and elimination after i.m. administration in neonatal calves (Gaur *et al.*, 2002) and calves (Mohan and Garg, 2002). However, disposition of ofloxacin has been described by a two-compartment open model following a single
intravenous administration in neonatal calves (Gaur et al., 2004).

Various pharmacokinetic parameters of ofloxacin derived from plasma concentration values following a single intramuscular injection in buffalo calves are given in Table 1. The absorption rate constant (ka) was $3.70 \pm 0.27$ h$^{-1}$ with an absorption half-life ($t_{1/2ka}$) of $0.19 \pm 0.02$ h, whereas elimination rate constant ($\beta$) was found to be $0.37 \pm 0.02$ h$^{-1}$ with an elimination half-life ($t_{1/2\beta}$) of $1.91 \pm 0.08$ h. However, higher values of $t_{1/2\beta}$ in calves ($3.11$ h) and neonatal calves ($16.61$ h) have been reported (Mohan and Garg, 2002; Gaur et al., 2002). The total body clearance ($Cl_{B}$) was $0.25 \pm 0.02$ L.kg$^{-1}$.h$^{-1}$ with an apparent volume of distribution ($V_{z(area)}$) of $0.69 \pm 0.03$ L.kg$^{-1}$ indicating moderate to good distribution and tissue penetration of ofloxacin following i.m. administration in buffalo calves. This is in contrast to the high values of $V_{z(area)}$ reported in calves ($1.39$ L.kg$^{-1}$) and neonatal calves ($4.39$ L.kg$^{-1}$) by Mohan and Garg (2002) and Gaur et al. (2002), respectively.

The AUC value ($12.38$ $\mu$g.ml$^{-1}$.h) was higher than the reported value of AUC ($7.96$ $\mu$g.ml$^{-1}$.h) in calves (Mohan and Garg, 2002). The values of AUMC and MRT were $36.12$ $\mu$g.ml$^{-1}$.h$^2$ and 2.90 h. Significantly higher values of MRT have been reported in calves and neonatal calves (Gaur et al., 2002; Mohan and Garg, 2002). Much higher values of AUMC ($943.7$ $\mu$g.ml$^{-1}$.h$^2$) and MRT ($24.03$ h) have been reported in neonatal calves (Gaur et al., 2002). The lower value of MRT in the present study also confirmed the presence of ofloxacin for a short duration (6 h) in plasma.

The maximum plasma concentration ($C_{max}$) was $3.78$ $\mu$g.ml$^{-1}$ observed at 30 minutes ($t_{max}$). The duration of therapeutic concentration ($t_{Cp(ther)}$) was computed to be 7.54 h following i.m. administration for maintaining MIC of $0.5$ $\mu$g.ml$^{-1}$ in buffalo calves.

The dosage regimen of ofloxacin was computed in male buffalo calves following i.m. administration to be a loading dose ($D^0$) of 6.51 mg.kg$^{-1}$ followed by a maintenance dose ($D^*$) of 6.16 mg.kg$^{-1}$ at a dosage interval (t) of 8 h which is sufficient to maintain therapeutic concentration of $0.5$ $\mu$g.ml$^{-1}$.

The concentrations of ofloxacin in urine were remarkably high and the drug was detected up to 48 h in the urine of buffalo calves following i.m. administration (Table 2). During the first two intervals of 3 h each, 142.49 mg and 120.74 mg of the drug was excreted which accounted for about 36 percent of the total drug administered. The amount of the drug excreted in urine during the first 12 h constituted a major part (91.72%) of the total drug excreted in the urine up to 48 h. Thereafter, less than 5 percent of the total administered drug was excreted in urine, i.e between 12 to 48 h. The cumulative percent of total dose excreted was 54.66 percent in 48 h (Table 2).

The total amount of ofloxacin excreted in urine in 48 h in the present study was 412.12 mg, i.e. 54.66 percent of the total drug administered. This is similar to the observations in pigs where 57.1% recovery of ofloxacin in urine after 10 mg.kg$^{-1}$ dose and 58.2% after 30 mg.kg$^{-1}$ dose following single intravenous bolus dose was found (Son et al., 2000). However, there was high recovery of ofloxacin in urine of pigs (74.2%) with a lower dose of 3 mg.kg$^{-1}$ (Son et al., 2000). Significantly high recovery of ofloxacin in human urine (72 to 98.5%) in unchanged form within 48 h has also been reported after single total dose of 100 to 600 mg (Ichihara et al., 1984; Verho et al., 1985; Lockley, 1988; Wise and Lockley, 1988). Our findings revealed that kidneys sequestered a major portion of the total drug following absorption and distribution from the site of intramuscular administration, and thus, the excretion of drug through urine was very high. Though the drug could not be detected in plasma 6 h after its administration, its excretion in urine continued up to 48 h. The high excretion of ofloxacin in urine of male buffalo calves indicated a strong ability of kidneys to concentrate ofloxacin in urine. This also correlated to its shorter elimination half-life ($t_{1/2\beta}$: $2.06 \pm 0.12$ h); this may be due to rapid excretion of the drug from the central compartment through urine. The results of this urinary excretion study of ofloxacin suggested that single i.m. injection of ofloxacin 5 mg.kg$^{-1}$ on alternate days will be efficacious if used in buffalo calves in the treatment of urinary tract infections sensitive to the drug.
Table 1. Plasma concentration and pharmacokinetic parameters of ofloxacin in male buffalo calves following single intramuscular injection of 5 mg.kg\(^{-1}\) b.wt.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Plasma concentration (µg.ml(^{-1}))</th>
<th>Parameter</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.55 ± 0.05</td>
<td>A(^b)</td>
<td>µg.ml(^{-1})</td>
<td>2.83 ± 0.18</td>
</tr>
<tr>
<td>10</td>
<td>2.92 ± 0.05</td>
<td>B</td>
<td>µg.ml(^{-1})</td>
<td>4.82 ± 0.16</td>
</tr>
<tr>
<td>15</td>
<td>3.24 ± 0.04</td>
<td>k(_a)</td>
<td>h(^{-1})</td>
<td>3.70 ± 0.27</td>
</tr>
<tr>
<td>30</td>
<td>3.78 ± 0.07</td>
<td>β</td>
<td>h(^{-1})</td>
<td>0.37 ± 0.02</td>
</tr>
<tr>
<td>45</td>
<td>3.32 ± 0.05</td>
<td>t(_{1/2\alpha})</td>
<td>h</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>60</td>
<td>3.09 ± 0.05</td>
<td>t(_{1/2\beta})</td>
<td>h</td>
<td>1.91 ± 0.08</td>
</tr>
<tr>
<td>90</td>
<td>2.82 ± 0.06</td>
<td>V(_{z(area)})</td>
<td>L.kg(^{-1})</td>
<td>0.69 ± 0.03</td>
</tr>
<tr>
<td>120</td>
<td>2.28 ± 0.05</td>
<td>AUC</td>
<td>µg.ml(^{-1}).h</td>
<td>12.40 ± 0.37</td>
</tr>
<tr>
<td>180</td>
<td>1.65 ± 0.04</td>
<td>AUMC</td>
<td>µg.ml(^{-1}).h(^2)</td>
<td>36.12 ± 2.19</td>
</tr>
<tr>
<td>240</td>
<td>1.10 ± 0.07</td>
<td>MRT</td>
<td>h</td>
<td>2.90 ± 0.11</td>
</tr>
<tr>
<td>360</td>
<td>0.54 ± 0.04</td>
<td>Cl(_B)</td>
<td>µg.ml(^{-1}).h(^{-1})</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>540</td>
<td>-</td>
<td>C(_{max})</td>
<td>µg.ml(^{-1})</td>
<td>3.72 ± 0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t(_{max})</td>
<td>min</td>
<td>42.19 ± 1.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t(_{cp(ther)})</td>
<td>h</td>
<td>7.54 ± 0.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D(^0)</td>
<td>mg.kg(^{-1})</td>
<td>6.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D(^*)</td>
<td>mg.kg(^{-1})</td>
<td>6.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t</td>
<td>h</td>
<td>8</td>
</tr>
</tbody>
</table>

Values are mean ± SE of six animals.

\(=\) Nondetectable

Ab, B = Zero-time plasma drug concentration intercepts of absorption and elimination phases, respectively; k\(_a\) = absorption rate constant; β = overall elimination rate constant; t\(_{1/2\alpha}\), t\(_{1/2\beta}\) = absorption and elimination half-lives, respectively; V\(_{z(area)}\) = apparent volume of distribution; AUC = area under plasma drug concentration-time curve; AUMC = area under first moment of plasma drug concentration-time curve; MRT = mean residence time; Cl\(_B\) = total plasma clearance; C\(_{max}\) = calculated maximum plasma concentration; t\(_{max}\) = time at which maximum plasma concentration achieved; t\(_{cp(ther)}\) = duration of therapeutic plasma drug concentration; D\(^0\) = loading dose; D\(^*\) = maintenance dose; t = dosage interval

Table 2. Urinary concentration and cumulative percent excretion of total dose of ofloxacin in male buffalo calves following single intramuscular injection of 5 mg.kg\(^{-1}\) b.wt.

<table>
<thead>
<tr>
<th>Time interval (h)</th>
<th>Urine concentration (µg.ml(^{-1}))</th>
<th>Cumulative percent excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 3</td>
<td>316.66 ± 19.72</td>
<td>18.93 ± 0.53</td>
</tr>
<tr>
<td>3 – 6</td>
<td>276.89 ± 32.33</td>
<td>34.96 ± 0.85</td>
</tr>
<tr>
<td>6 – 9</td>
<td>180.66 ± 8.69</td>
<td>44.51 ± 1.15</td>
</tr>
<tr>
<td>9 – 12</td>
<td>102.99 ± 11.52</td>
<td>50.16 ± 1.30</td>
</tr>
<tr>
<td>12 – 24</td>
<td>17.77 ± 1.94</td>
<td>53.38 ± 1.51</td>
</tr>
<tr>
<td>24 – 36</td>
<td>4.50 ± 0.52</td>
<td>54.33 ± 1.57</td>
</tr>
<tr>
<td>36 – 48</td>
<td>1.72 ± 0.20</td>
<td>54.66 ± 1.56</td>
</tr>
</tbody>
</table>

Values are mean ± SE of six animals.

\(=\) Nondetectable
REFERENCES


*Continued on page 164
Harbour Laboratory, Cold Spring Harbour, New York.


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