Pharmacokinetics of levofloxacin after oral administration in normal and ketoprofen-treated sheep

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Abstract
The pharmacokinetics of levofloxacin was evaluated in female sheep following its single oral administration (3 mg kg\(^{-1}\)) alone and on concomitant intramuscular (i.m.) administration of ketoprofen (3 mg kg\(^{-1}\)). Levofloxacin concentration was determined using the High Performance Liquid Chromatography. Following oral administration of the drug, mean absorption rate constant (K\(_a\)) and mean elimination rate constant (\(\beta\)) were 1.21 ± 0.19 and 0.13 ± 0.01 h\(^{-1}\), respectively. The absorption (t\(_{\text{abs}}\)) and elimination half-life (t\(_{\text{1/2}}\)) were 0.75 ± 0.23 and 5.25 ± 0.21 h, respectively. The mean apparent volume of distribution (\(V_{\text{d(app)}}\)), area under plasma drug concentration-time curve (AUC\(_{\text{0-\infty}}\)) and area under first moment curve (AUMC) were 4.35 ± 0.29 L/kg, 2.78 ± 0.21 µg.h/mL and 22.54 ± 1.14 µg.h\(^2\)/mL, respectively. The mean value of total body clearance (Cl\(_b\)) of the drug was 0.55 ± 0.01 L/h/kg with mean residence time (MRT) of 8.18 ± 0.28 h. The calculated mean value of mean absorption time (MAT) was 6.45 ± 0.26 h. The levofloxacin concentrations from 8 to 24 hrs were significantly higher when ketoprofen was administered along with levofloxacin. However, pharmacokinetic parameters of orally administered levofloxacin were not significantly affected by concomitant administration of ketoprofen in sheep. Oral administration of levofloxacin at 3 mg/kg in sheep resulted in lower concentrations overall thus the drug may be efficacious against bacteria with lower MIC value (0.03 µg/mL).

Keywords: Pharmacokinetics, levofloxacin, ketoprofen, oral administration, sheep

Introduction
Antibacterial drugs are employed for the treatment of bacterial diseases in animals. Tetracyclines, cephalosporins, quinolones are important groups of drugs which are used for the treatment of mixed infection in animals. Pharmacological aspects of cephalosporins are studied in animals particularly pharmacokinetic profiles are extensively evaluated (Tiwari et al., 2009; Patel et al., 2006\(^a\); Patani et al., 2006; Gohil et al., 2009; Maradiya et al., 2010; Patel et al., 2010; Swati et al., 2010) \[13, 30, 26, 23, 10, 17, 31, 30\] and many of them are now used in animals. However, due to constrain of bacterial resistance, the newer generation of cephalosporines are kept for limited uses in animals. Newer generation fluoroquinolones are extensively evaluated for having good pharmacokinetics in animals and birds (Patel et al., 2011; Modi et al., 2012) \[25, 18\]. Amongst them, levofloxacin is newer generation fluoroquinolone which is active against Gram-positive aerobic organisms, Gram-negative bacteria and anaerobes (Davis and Bryson, 1994; Klesel et al., 1995) \[14, 15\]. The potential value of levofloxacin was described by previous studies on its pharmacokinetic profiles in various species of domestic animals and birds (Gonzalez et al., 2001; Albarellos et al., 2005; Patel et al., 2012\(^{bc}\); Varia et al., 2009; Varia et al., 2012; Patel et al., 2013\(^{b}\)) \[11, 1, 23, 26, 29, 38, 37\]. Many studies have been done to evaluate pharmacokinetic profile of levofloxacin following intravenous, intramuscular or subcutaneous administration in animals. Safety profile of levofloxacin has also been studied in sheep and poultry (Patel et al., 2009; Patel et al., 2013\(^{ab}\)) \[24, 22, 27\]. In addition, concomitant use of NSAIDs may invariably affect disposition of the quinolones (Shiba et al., 1992) \[34\] with enhancement of the convulsant activity of quinolone. The alteration in disposition of levofloxacin on concomitant administration of paracetamol and meloxicam in calves has been reported previously (Dumka et al., 2007\(^{ab}\); Dumka, 2007) \[6, 7, 5\]. Ketoprofen (KTP), an aryl propionic acid derivative, non-selective COX inhibitor NSAID which is useful as anti-inflammatory, analgesic and antipyretic drug for the treatment of inflammatory conditions including rheumatoid arthritis and osteoarthritis in animals (Green, 2001; Owens et al. 1995\(^{ab}\)) \[13, 19, 20\].
A pharmacokinetic profile of ketoprofen has been evaluated in animals (Landoni et al., 1999; Ratndee Singh et al., 2014) [16, 32].

Data on pharmacokinetics of levofloxacin in small ruminants like sheep following oral administration are not available. The pharmacokinetic interaction of levofloxacin along with ketoprofen administration has not been studied in sheep. Thus, present study was carried out to evaluate the pharmacokinetics of levofloxacin following oral administration in sheep with possible pharmacokinetic interaction with ketoprofen.

Material and Methods

Experimental animals

The experiment was conducted on six healthy female Patanwadi non-lactating sheep of 2-3 years old age ranging in body weight from 23.5 to 30.0 kg obtained from and maintained at the Instructional Farm, College of Veterinary Science and Animal Husbandry, AAU, Anand, India. Constant observation for two weeks prior to commencement of the experiment was followed with clinical examination in order to exclude the possibility of any disease. The experimental protocol of the present study has been approved by the Animal Ethics Committee.

Drug administration and sampling

Levofloxacin oral tablet were procured from local pharmacy. Levofloxacin tablet (250 mg) was dissolved in 25 mL sterile water and used for oral administration using a syringe without the needle. Animals were fasted for 24 h before the oral administration of the drug. Ketoprofen was administered at the dose rate of 3 mg/kg of body weight intramuscularly in deep gluteal muscle. The washout period of 15 days was observed between two treatments of levofloxacin alone and levofloxacin with ketoprofen to rule out possibility of drug residue. Blood samples (3 mL) were collected from i.v. catheter (Venflon, 22 × 0.9 × 25 mm) fixed into the right jugular vein into heparinized centrifuge tube. Following oral administration of the drug, blood samples were collected at 0 (prior to treatment), 0.083, 0.166, 0.33, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 18, 24, 36 and 48 h post-treatment. Plasma was separated soon after collection by centrifugation at 3000 g for 15 min and transferred to labeled cryovials and stored at -35 °C until assayed for levofloxacin concentration using high performance liquid chromatography (HPLC) procedure which was usually done within 24 to 36 h.

Levofloxacin assay

The high performance liquid chromatography apparatus of Laballiance (USA) comprising quaternary gradient delivery pump (model AIS 2000) and UV detector (model 500) was used for assay. Chromatographic separation was performed by using reverse phase C18 column (Thermo, ODS; 250 × 4.6 mm ID) at room temperature. The HPLC data integration was carried out to evaluate the pharmacokinetic parameters in ketoprofen treated and normal sheep according to Snedecor and Cochran (1980) [38].

PK/PD integration

Efficacy predictors like Cmax/MIC90 and AUC0-∞/MIC90 for concentration dependent antibiotic levofloxacin were calculated using the values of peak plasma drug concentration (Cmax) and area under the curve (AUC0-∞) after oral administration. MIC90 data of levofloxacin against ovine bacterial isolates have not been reported earlier. Thus, to cover most of the susceptible organisms like Klebsiella spp., Shigella spp. Salmonella spp., Proteus spp. and Acinetobacter spp. in this discussion, the MIC90 of 0.12 μg mL⁻¹ of levofloxacin has been taken into consideration as described by Goudah and Hasabelnabhy (2010) [12].

Result and Discussion

Following oral administration of the levofloxacin, the drug concentration of 0.026 ± 0.005 μg/mL was observed at 0.5 h. The mean peak plasma drug concentration of 0.779 ± 0.028 μg/mL was achieved at 4 h which declined rapidly to 0.147 ± 0.005 μg/mL at 12 h. The drug concentration of 0.018 ± 0.001 μg/mL in plasma was detected at 24 h. The drug was not detected in plasma samples collected after 24 h post oral administration of levofloxacin in sheep. Following oral administration of the levofloxacin along with ketoprofen, the levofloxacin concentration of 0.028 ± 0.003 μg/mL was observed at 0.5 h. The mean peak plasma drug concentration of 0.768 ± 0.030 μg/mL was achieved at 4 h which declined rapidly to 0.125 ± 0.012 μg/mL at 12 h. The drug concentration of 0.030 ± 0.003 μg/mL in plasma was detected at 24 h. The drug was not detected in plasma samples collected after 24 h post oral administration of levofloxacin with ketoprofen in sheep. The drug concentration-time profile of levofloxacin in normal and ketoprofen-treated sheep is tabulated in Table 1 and graphically depicted in Figure 1. Pharmacokinetic parameters of levofloxacin following oral administration in normal and ketoprofen-treated sheep are as per Table 2.

Following oral administration of levofloxacin in normal sheep, peak plasma drug concentration (Cmax) observed at 4 h (Tmax) is found to be lower than Cmax of 4.38 ± 1.52 μg/mL at 1.18 h following oral administration (10 mg/kg) in cats (Albarrelos et al., 2005) [11]. It indicates slower and poor absorption of the drug following oral administration in sheep compared to cats. However, Cmax of norfloxacin (1.28 ± 0.26 μg/mL) was also observed at 10.20 ± 3.98 h following oral administration (60 mg/kg) in sheep (Gonzaalaz et al., 2001) [11]. Significant higher (p<0.05) plasma drug concentrations were also obtained in ketoprofen-treated sheep (8 to 24 h) compared to respective values in normal sheep. The last observed drug concentrations in both ketoprofen-treated sheep were significantly higher than the respective value in normal sheep.

Following single dose oral administration of levofloxacin in normal sheep, the absorption rate constant (Ka) and half-life (t1/2Ka), were found to be 1.21 ± 0.19 h⁻¹ and 0.75 ± 0.23 h, respectively. The respective values for levofloxacin in ketoprofen-treated sheep were not significantly altered (p>0.05). Absorption half-life of 1.08 ± 0.05 h has been observed after oral administration of levofloxacin in chicken (Patel et al., 2012) [23].
to that of normal sheep. As fluoroquinolones have high lipid solubility and low plasma protein binding they are widely distributed in body and the same has been observed in the present study. In most species, the distribution volume of levofloxacin and other fluoroquinolones is greater than that for most β-lactam antibiotics and aminoglycosides, and probably represents intracellular sequestration of the drug in various tissues (Brown, 1996) [2]. The values of AUC, AUMC, \( \text{Vd}_0 \) of levofloxacin following oral administration were not significantly altered by administration of ketoprofen in sheep.

Following oral administration of levofloxacin relatively longer elimination half-life of 8.37 ± 3.47 h in cats (Albarellos et al., 2005) [1], 3.62 ± 0.12h in chickens (Patel et al, 2012) [21], 5.65 ± 0.14 h in mice (Ender et al., 2003) [8], 7.1 h (Chien et al., 1997) [13] and 4.23 ± 0.87 h (Hilte et al., 2000) [14] in human have also been reported. Elimination half-life (\( t_{1/2b} \)) of the drug following oral administration is higher than \( t_{1/2a} \) after intravenous administration (Patel et al, 2012) [20]; this means drug is likely to act longer time after oral administration. Half-life depends on urinary pH of animal, and so may be different in various species. It also depends on volume of distribution and clearance of the drug and in the present study, clearance was found lower. So, half-life was found to be high, as it is inversely proportional to clearance.

The bioavailability of levofloxacin after oral administration in normal and ketoprofen-treated sheep was found to be similar. Higher bioavailability of levofloxacin following oral administration has been reported in cats (71.20 ± 22.99 %) and chickens (71.61 ± 1.38 %) (Albarellos et al., 2005; Patel et al, 2012) [1, 21]. Levofloxacin is rapidly absorbed from the gastrointestinal tract with absolute bioavailability after oral administration in humans. Administration of levofloxacin with food slows its absorption, prolongs peak serum concentrations by 1 h, and decreases the peak serum concentration by 14% (Fish and Chow, 1997) [9]. Moderate to low bioavailability in the present study indicates moderate absorption of levofloxacin via gastrointestinal tract after oral administration in normal and ketoprofen-treated sheep. Moreover, therapeutically effective concentration produced and maintained for up to 24 hours suggest that oral administration of levofloxacin may be conventional for the treatment of systemic bacterial infections in sheep. However, bacteria with lower MIC values can be effectively eradicated with levofloxacin administration by oral route in sheep.

### Table 1: Comparison of plasma concentrations (µg/mL) of levofloxacin after oral administration (3 mg/kg) in normal and ketoprofen-treated (3 mg/kg) sheep

<table>
<thead>
<tr>
<th>Time after drug administration (h)</th>
<th>(Normal)</th>
<th>(Ketoprofen-treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.026 ± 0.005</td>
<td>0.028 ± 0.003</td>
</tr>
<tr>
<td>0.75</td>
<td>0.044 ± 0.006</td>
<td>0.051 ± 0.007</td>
</tr>
<tr>
<td>1</td>
<td>0.091 ± 0.002</td>
<td>0.145 ± 0.011*</td>
</tr>
<tr>
<td>2</td>
<td>0.291 ± 0.028</td>
<td>0.256 ± 0.003</td>
</tr>
<tr>
<td>4</td>
<td>0.779 ± 0.028</td>
<td>0.768 ± 0.030</td>
</tr>
<tr>
<td>6</td>
<td>0.291 ± 0.016</td>
<td>0.306 ± 0.036</td>
</tr>
<tr>
<td>8</td>
<td>0.147 ± 0.005</td>
<td>0.207 ± 0.020*</td>
</tr>
<tr>
<td>12</td>
<td>0.072 ± 0.004</td>
<td>0.125 ± 0.012**</td>
</tr>
<tr>
<td>18</td>
<td>0.033 ± 0.001</td>
<td>0.050 ± 0.005**</td>
</tr>
<tr>
<td>24</td>
<td>0.018 ± 0.001</td>
<td>0.030 ± 0.003**</td>
</tr>
<tr>
<td>36</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: Not Detected; *Significant at \( p<0.05 \), **Highly significant at \( p<0.01 \) when compared with respective values of normal sheep.

### Table 2: Pharmacokinetic parameters (Mean ± S.E.) of levofloxacin after oral administration (3 mg/kg) in normal and ketoprofen-treated (3 mg/kg) sheep

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Unit</th>
<th>(Normal)</th>
<th>(KTP-treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>µg/mL</td>
<td>0.67 ± 0.14</td>
<td>1.08 ± 0.23</td>
</tr>
<tr>
<td>B</td>
<td>µg/mL</td>
<td>0.41 ± 0.05</td>
<td>0.50 ± 0.03</td>
</tr>
<tr>
<td>Kₐ</td>
<td>h⁻¹</td>
<td>1.21 ± 0.19</td>
<td>1.32 ± 0.26</td>
</tr>
<tr>
<td>β</td>
<td>h⁻¹</td>
<td>0.13 ± 0.01</td>
<td>0.14 ± 0.003</td>
</tr>
<tr>
<td>t½Ka</td>
<td>h</td>
<td>0.75 ± 0.23</td>
<td>0.61 ± 0.14</td>
</tr>
<tr>
<td>t½β</td>
<td>h</td>
<td>5.25 ± 0.21</td>
<td>4.82 ± 0.11</td>
</tr>
<tr>
<td>Cₘax</td>
<td>µg/mL</td>
<td>0.78 ± 0.03</td>
<td>0.77 ± 0.03</td>
</tr>
<tr>
<td>Tₘax</td>
<td>h</td>
<td>4.00 ± 0.00</td>
<td>4.00 ± 0.00</td>
</tr>
<tr>
<td>AUC(0, ∞)</td>
<td>µg.h/mL</td>
<td>2.78 ± 0.21</td>
<td>2.79 ± 0.11</td>
</tr>
<tr>
<td>AUMC</td>
<td>µg.h²/mL</td>
<td>22.54 ± 1.14</td>
<td>22.92 ± 0.71</td>
</tr>
<tr>
<td>Vd(ave)</td>
<td>L/kg</td>
<td>4.35 ± 0.29</td>
<td>3.99 ± 0.19</td>
</tr>
<tr>
<td>Cl(ave)</td>
<td>L/h/kg</td>
<td>0.55 ± 0.01</td>
<td>0.57 ± 0.01</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>8.18 ± 0.28</td>
<td>7.57 ± 0.46</td>
</tr>
<tr>
<td>MAT</td>
<td>h</td>
<td>6.45 ± 0.26</td>
<td>5.90 ± 0.44</td>
</tr>
<tr>
<td>F</td>
<td>%</td>
<td>52.48 ± 2.52</td>
<td>52.97 ± 1.88</td>
</tr>
</tbody>
</table>

*Significant at \( p<0.05 \), compared with respective values of normal sheep.
Conclusion
Pharmacokinetic profiles of levofloxacin in normal sheep were found moderate as compared to pharmacokinetic profiles of the drug following parental route of administration. Ketoprofen administration did not affect the oral pharmacokinetic profile of levofloxacin in sheep. After oral administration, PK/PD efficacy predictors indicated that oral administration of levofloxacin (3 mg/kg) in normal and ketoprofen-treated sheep would be efficacious against bacteria with low MIC of 0.03 µg/mL only or higher dose may be needed to overcome lesser extent of absorption following oral administration in sheep.

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References


