Effect of flunixin co-administration on pharmacokinetics of cefquinome following intramuscular administration in goats

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Abstract
Cefquinome, a fourth generation cephalosporin, having broad spectrum of activity against Gram-positive and Gram-negative bacteria and developed exclusively for veterinary use. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used with antimicrobial therapy in veterinary practice for the treatment of infectious diseases. The present study was taken to investigate the disposition kinetics of cefquinome at the dose rate of 2 mg kg\(^{-1}\) body weight and the effect of flunixin when co-administered intravenously at the dose rate of 2.2 mg kg\(^{-1}\) body weight on disposition kinetics of cefquinome in goats. Plasma concentrations were determined by microbiological assay method using MTCC equivalent (MTCC 1541) of Micrococcus luteus (ATCC 9341) as the test organism. Following intramuscular administration of cefquinome, the plasma concentration time profile was best described by one compartment open model. In the present study, following intramuscular administration of cefquinome in flunixin pretreated goats, the peak plasma concentration (C\(_{\text{max}}\)) was observed 4.48 ± 0.19 μg ml\(^{-1}\) which was not significantly higher and almost comparable to the C\(_{\text{max}}\) 5.1 ± 0.51 μg ml\(^{-1}\) observed in only cefquinome administered goats. Following co-administration of cefquinome with flunixin, no significant changes were observed in most of the pharmacokinetic parameters of cefquinome, except elimination half-life (t\(_{1/2\beta}\)) and volume of distribution (Vd\(_{\text{area}}\)) which were significantly increased. The integration of pharmacokinetic and pharmacodynamics parameters suggested that the cefquinome at the dose rate of 2 mg kg\(^{-1}\) body weight intramuscularly can be administered with flunixin to treat bacterial infections with inflammatory conditions in goats.

Keywords: Disposition kinetic, Microbiological assay, compartmental open model

Introduction
Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly indicated as an adjunct to antimicrobial therapy in veterinary practice for the treatment of infectious diseases. Cefquinome is a fourth generation aminothiazolyl cephalosporin with broad spectrum of activity against Gram-positive and Gram-negative bacteria, developed exclusively for veterinary use including food animals (Murphy et al., 1994)\(^{[16]}\). It has a broad spectrum of antibacterial activity with time dependent bactericidal effect, as shown by β-lactam antibiotics (Thomas et al., 2006)\(^{[7]}\). In ruminants, the use of NSAIDs is associated with the treatment of pain, mastitis, pneumonia and inflammatory conditions (Pugh, 1991; Ziv, 1992; Deleforge et al., 1994)\(^{[20, 32, 7]}\). Amongst Non-steroidal anti-inflammatory drugs, Flunixin, used for analgesic, antiphlogistic and antipyretic purposes in a variety of mammalian species. Flunixin acts via analgesic and anti-inflammatory mechanisms. Analgesic actions may involve blocking pain impulse generation via a peripheral action by inhibition of the synthesis of prostaglandins and possibly inhibition of the synthesis or actions of other substances, which sensitize pain receptors to mechanical or chemical stimulation. Flunixin may act peripherally in inflamed tissue, probably by inhibiting the enzyme cyclooxygenase to decrease the formation of precursors of prostaglandins, and possibly by inhibiting other local mediators of the inflammatory response (Lees and Higgins, 1985)\(^{[14]}\). Various pharmacokinetic interactions between antimicrobials and NSAIDs have been described (Singh et al., 2008; Dumka et al., 2010; Abo-El-Sooud and AL-Anati, 2011; Ranjan et al., 2011; Ali and Mohiuddin, 2012; Patel et al., 2012a; Patel et al., 2012b)\(^{[24, 8, 1, 22, 4, 17, 18]}\). There is less information available on the influence of the co-administration of flunixin on the pharmacokinetics of cefquinome in animals. So, the present study was conducted to investigate the influence of flunixin on the pharmacokinetics of cefquinome in goats.
Material and Method

Experimental animals

The study was conducted on five apparently healthy Marwari male goats of 8 – 10 month of age (Weight between 25-30 kilograms). The experiment was conducted at the Livestock Research Station, Kodamdesar, Rajasthan University of Veterinary and Animal science, Bikaner. During this period they were subjected to clinical examination in order to exclude the possibility of any disease. The animals were then housed in separate pens and maintained on concentrate, green fodder and water ad libitum. The experimental protocol for general procedure and use of animals for conducting the present study has been reviewed and approved by the Institutional Animal Ethics Committee (IAEC) and submitted to CPCSEA.

Drugs and test organism

Cefquinome sulphate injection (25 mg ml⁻¹; Cobactan 2.5%, MSD Healthcare) was purchased from local market. Flunixin injection (Flunimeg, Zydas Animal Health) was purchased from local market. MTCC equivalent (MTCC 1541) of Micrococcus luteus (ATCC 9341) as the test organism used in microbiological assay work for the present study, were procured from institute of Microbial Technology (IMTECH), Chandigarh, India.

Experimental protocol

The study was carried out in cross-over design, with a minimum of 15 days of washout period. Cefquinome sulphate injection was administered intramuscularly on gluteal muscle at the dose rate of 2 mg kg⁻¹ body weight. The dosage level of cefquinome employed in the present study was comparable to the dose of cefquinome used by previous workers in goat (Dumka et al., 2013) [9] and sheep (Uney et al., 2011) [29]. Blood samples were collected in test tubes containing EDTA, immediately before administration of cefquinome (0 h) and at 0.08, 0.16, 0.33, 0.5, 0.75, 1.0, 1.5, 2, 4, 6, 8, 10, 12 and 24 h after administration of the drug. Following a washout period of 21 days, Cefquinome sulphate injection at the dose rate of 2 mg kg⁻¹ body weight intramuscularly and flunixin injection at the dose rate of 2.2 mg kg⁻¹ body weight intravenously were administered simultaneously. Blood samples were collected before administration of cefquinome and flunixin (0 h) and at same time intervals mentioned above after administration of the drugs. Blood samples were centrifuged at 3000 rpm for 15 min to separate the plasma. Plasma samples were stored at −20 °C until assayed.

Drug bioassay

Cefquinome concentrations in plasma samples were estimated by microbiological assay method using MTCC equivalent (MTCC 1541) of Micrococcus luteus (ATCC 9341) (Arret et al., 1971; El Badawy et al., 2015) [5, 10]. Six equidistant identical (6.0±0.1 mm) wells were punched in the solidified media in petri plates using a punching machine. The wells were charged with the test samples/cefquinome standard in triplicates. The petri plates were incubated at 30 degree C for 24 h. In test sample plates, three alternate wells out of six were filled with reference concentration of the drug which gives a clear zone of inhibition of 16-18 mm so as to minimize the plate to plate variation in zones of inhibition. Zones of inhibition were measured using a Vernier calliper and the mean of triplicate samples was taken and compared with that of reference standard(s) to obtain the concentration of cefquinome in test samples. Minimum sensitivity of the assay method was 0.20 µg ml⁻¹.

Pharmacokinetic analysis

The plasma cefquinome concentration time profile of each animal following intramuscular administration of cefquinome alone and when co-administered with flunixin were used to determine the pharmacokinetic variables describing the absorption, distribution and elimination characteristics of cefquinome in goats. To determine the different disposition kinetic variables, plasma drug concentration–time data were analyzed by employing the compartmental (Gibaldi and Perrier, 2007; Baggot, 2001) [12, 6] and non-compartmental (Yamoka et al., 1978; Gibaldi and Perrier, 2007) [30, 12] pharmacokinetic models.

Statistical analysis of data

The data generated in the present studies were subjected to statistical analysis by employing student’s ‘t’ test using MS Excel (2007).

Result and discussion

No adverse effects or toxic manifestations were observed to cefquinome IM injection alone or co-administered with flunixin in goats. Plasma cefquinome concentrations at different time intervals following intramuscular administration of cefquinome alone and co-administered with flunixin in goats is presented as semi logarithmic plot in Figure-1 and Figure-2 respectively. Following intramuscular administration of cefquinome at the dose rate of 2 mg kg⁻¹ body weight in goats, the plasma concentration of cefquinome was quickly raised well above minimum inhibitory concentration (MIC) against pathogens (Ahmad et al., 2015) and found 0.72 ± 0.28 µg ml⁻¹ was observed at 0.08 h after drug administration. The peak plasma concentration (C_max) was observed 5.10 ± 0.51 µg ml⁻¹ at 1.00 h post administration of cefquinome in goats at 2 mg kg⁻¹ body weight. In the present study, the higher peak plasma concentration (C_max) was observed than in 4.36 ± 0.10 µg ml⁻¹ at 0.75 h in sheep (Rana et al., 2015) [31], 4.84 ± 0.23 µg ml⁻¹ at 1.50 h in goat (Dumka et al., 2013) [9], 4.01 ± 0.57 µg ml⁻¹ in piglets (Li et al., 2008) [13], and 4.83 µg ml⁻¹ at 0.43 h in dog (Zhou et al., 2015) [33] administered cefquinome intramuscularly at the same dose. However, the Higher value of the C_max like 9.05 ± 0.06 µg ml⁻¹ at 0.95 h in rabbits (Shalaby et al., 2014) [23] and 9.38 ± 1.69 µg ml⁻¹ at 0.38 h in duck (Yuan et al., 2011) [31], C_max achieved earlier in sheep (Rana et al., 2015) [31], dog (Zhou et al., 2015) [33], rabbit (Shalaby et al., 2014) [23] and duck (Yuan et al., 2011) [31]. While later in goats (Dumka et al., 2013) [9] and camel (Al-Taher, 2010) [9].
In the present study, following intramuscular administration of cefquinome in flunixin pretreated goats, the peak plasma concentration ($C_{\text{max}}$) was observed $4.48 \pm 0.19 \mu g \text{ ml}^{-1}$ which was not significantly higher and almost comparable to the $C_{\text{max}}$ 5.1 ± 0.51 μg ml$^{-1}$ observed in only cefquinome administered goats. Similarly, no significant alteration in $C_{\text{max}}$ of cefquinome following co-administration with meloxicam in sheep was found by Tiwari et al. (2015) [25] which supports the present study. However, in contrast to present study, a significant increase in peak plasma concentration ($C_{\text{max}}$) of cefquinome was observed in tolfenamic acid co-administrated sheep (4.73 ± 0.05 μg ml$^{-1}$) as compared to cefquinome alone treated sheep (4.36 ± 0.10 μg ml$^{-1}$) by Rana et al. (2015) [21]. Likewise EL-Hewaity (2014) [11] also reported a significant increase in $C_{\text{max}}$ in goats following cefepime co-administration with flunixin as compared to cefepime alone treated goats. Similarly, $C_{\text{max}}$ of cefepime was also found higher in ketoprofen administered sheep than $C_{\text{max}}$ observed in cefepime alone (Patel et al., 2012b) [18]. Likewise Singh et al. (2008) [24] also reported significantly higher $C_{\text{max}}$ value of ceftiraxone given with the paracetamol as compared to ceftiraxone alone treated animals. Whereas the lower value of $C_{\text{max}}$ of ceftiraxone was found when coadministered with paracetamol (19 ± 0.32 μg ml$^{-1}$) in comparison ceftiraxone alone treated goats (45.6 ± 0.19 μg ml$^{-1}$) in findings of Jimoh et al. (2011) [13]. The disposition kinetics of cefquinome in goats could be described by one compartment open model considering the plasma concentration versus time semi logarithmic curve. Disposition of cefquinome has been described with one compartment open model in goats (Dumka et al., 2013) [9], dog (Zhou et al., 2015) [33] but two-compartment open model in sheep (Tohamy, 2011) [27], piglets (Li et al., 2008) [15] and duck (Yuan et al., 2011) [31]. The comparative pharmacokinetic parameters were generated following intramuscular administration of cefquinome alone and co-administered with flunixin and subjected to statistical analysis using students’s 't' test using MS Excel (2007), are depicted in the table 1.
Following intramuscular administration of cefquinome with flunixin in goats most of the pharmacokinetic parameters were not significantly altered in comparison to goats administered cefquinome alone except elimination half-life ($t_{1/2\beta}$) and volume of distribution ($V_{d(area)}$). The absorption half-life ($t_{1/2ka}$) of cefquinome co-administration with flunixin in goats was found to be 0.21 ± 0.02 h which is almost similar to $t_{1/2ka}$ 0.29 ± 0.04 h observed in cefquinome alone, suggesting co-administration of flunixin does not alter the absorption of cefquinome in goats. Similarly, no significant alteration was observed in absorption half-life ($t_{1/2ka}$) of cefquinome (0.15 ± 0.01 h to 0.16 ± 0.01 h) in goats when co-administration with the meloxicam (Tiwari et al., 2015) [25] which was in support to present study. Likewise, El-Hewaily (2014) [11] also reported no significant alteration in $t_{1/2ka}$ of cefepime (0.25 ± 0.02 h to 0.28 ± 0.03 h) in goats when co-administration with the flunixin. Also no significant alteration in $t_{1/2ka}$ of cefepime (0.27 ± 0.05 h to 0.18 ± 0.02 h) was observed when co-administered with ketoprofen in goats (Patel et al., 2012a) [17]. Similar result was found when absorption half-life ($t_{1/2ka}$) of cefepime (0.17 ± 0.01 h to 0.18 ± 0.01 h) in cow calves when co-administered with ketoprofen (Patil et al., 2012) [19]. Likewise, Singh et al. (2008) [24] also reported no significant alteration in $t_{1/2ka}$ value of ceftriaxone (0.23 ± 0.03 h to 0.21 ± 0.02 h) in cross bred calves when co-administration with paracetamol which also support the present study.

In contrast to present study, Jimoh et al. (2011) [13] reported significant decrease in the $t_{1/2ka}$ value of ceftriaxone in goats co-administered with paracetamol (0.17 ± 0.02 h) in comparison to given ceftriaxone (0.51 ± 0.004h) alone. Rana et al. (2015) [21] also reported significant decrease in $t_{1/2ka}$ of cefquinome co-administration with flunixin (0.26 ± 0.01) in comparison to given cefquinome (0.61 ± 0.10 h) alone. However, the significant increase $t_{1/2ka}$ value of cefepime (0.16 ± 0.01 h to 0.22 ± 0.001 h) was reported in sheep when co-administered with ketoprofen (Patel et al., 2012b) [18].

The elimination half-life ($t_{1/2\beta}$) of cefquinome co-administration with flunixin in goats was found 1.85 ± 0.12 h which is significantly higher to the $t_{1/2\beta}$ 1.48 ± 0.04 h observed in cefquinome alone treated goats. Similar significant increase in the value of $t_{1/2\beta}$ of ceftriaxone was found in cross bred calves when co-administration with paracetamol (4.08 ± 0.54 h) in comparison to given ceftriaxone alone (1.44 ± 0.12 h) by Singh et al. (2008) [24] which supports the present study.

Similarly, Jimoh et al. (2011) [13] also reported the higher $t_{1/2\beta}$ in goat given ceftriaxone co-administration with paracetamol (5.34 ± 1.85 h) in comparison to given ceftriaxone (0.58 ± 0.02 h) alone which also supports the present study. However, no significant alteration found in $t_{1/2\beta}$ of cefquinome co-administration with tolfenamic acid (9.00 ± 0.51 h) in comparison to given cefquinome alone (12.29 ± 2.62 h) by Rana et al. (2015) [21]. Significant alteration in $t_{1/2\beta}$ of cefepime was also not found in calves when co-administered with ketoprofen (5.36 ± 0.19 h) in comparison to given cefepime (5.15 ± 0.09 h) alone (Patil et al., 2012) [19]. No alteration in $t_{1/2\beta}$ of cefquinome in goats was found when co-administration with meloxicam (1.60 ± 0.05 h) in comparison to given cefquinome (1.75 ± 0.08 h) alone (Tiwari et al., 2015) [25]. Similarly, no significant alteration in $t_{1/2\beta}$ of cefepime was found in goats when co-administration with ketoprofen (5.32 ± 0.19 h) in comparison to given cefepime (5.13 ± 0.2 h) alone (Patel et al., 2012a) [17]. The area under the curve (AUC) of cefquinome when co-administration with flunixin in goats was 13.67 ± 0.57 μg ml⁻¹h which is almost similar to the AUC observed in goats given cefquinome alone 14.44 ± 0.82 μg ml⁻¹h. Similarly no significant alteration was found in the AUC of cefquinome in sheep when co-administration with tolfenamic acid (17.52 ± 0.14 μg ml⁻¹h) in comparison to cefquinome given (16.65 ± 0.57 μg ml⁻¹h) alone (Rana et al., 2015) [21]. Likewise, no significant alteration found in the AUC of cefquinome in goats when co-administration with meloxicam (17.16 ± 0.14 μg ml⁻¹h) in comparison to cefquinome given (18.49 ± 0.74 μg ml⁻¹h) alone which was in support to present study.

### Table 1: Pharmacokinetic parameters of cefquinome (2 mg kg⁻¹) following a single dose, intramuscular administration alone and in combination with flunixin (2.2 mg kg⁻¹) treated goats (Mean ± SE, n = 5) employing one compartment open model.

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Units</th>
<th>Mean±S.E.</th>
<th>Cefquinome alone</th>
<th>Cefquinome with Flunixin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ka</td>
<td>h⁻¹</td>
<td>2.67 ± 0.52</td>
<td>3.49 ± 0.31</td>
<td></td>
</tr>
<tr>
<td>$t_{1/2ka}$</td>
<td>h</td>
<td>0.29 ± 0.04</td>
<td>0.21 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>β</td>
<td>h⁻¹</td>
<td>0.47 ± 0.01</td>
<td>0.38 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>$t_{1/2\beta}$</td>
<td>h</td>
<td>1.48 ± 0.04</td>
<td>1.85 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Cmax(obs)</td>
<td>µg ml⁻¹</td>
<td>5.39 ± 0.38</td>
<td>4.57 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>$t_{max(obs)}$</td>
<td>h</td>
<td>1.15 ± 0.15</td>
<td>0.95 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>AUC</td>
<td>µg ml⁻¹h⁻¹</td>
<td>14.44 ± 0.82</td>
<td>13.67 ± 0.57</td>
<td></td>
</tr>
<tr>
<td>AUMC</td>
<td>µg ml⁻¹h⁻¹</td>
<td>38.19 ± 2.45</td>
<td>34.35 ± 4.13</td>
<td></td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>2.64 ± 0.07</td>
<td>2.56 ± 0.36</td>
<td></td>
</tr>
<tr>
<td>$V_{d(area)}$</td>
<td>L kg⁻¹</td>
<td>0.29 ± 0.02</td>
<td>0.42 ± 0.042**</td>
<td></td>
</tr>
<tr>
<td>CI’</td>
<td>ml kg⁻¹h⁻¹</td>
<td>140.33 ± 8.09</td>
<td>147.41 ± 6.44</td>
<td></td>
</tr>
</tbody>
</table>

NS: Non-significant; *: Significant at P<0.05 (95% confidence level); **: Significant at P<0.01 (99% confidence level)
ml⁻¹ h⁻¹) alone (Singh et al., 2008) [24]. The area under the moment curve (AUMC) of cefquinome when co-administration with flunixin in goats was (34.35 ± 4.13 μg ml⁻¹ h²) which is comparably similar to the AUMC (38.19 ± 2.45 μg ml⁻¹ h²) observed in cefquinome alone. Similarly, no alteration found in the AUMC of cefquinome in goats when co-administration with the meloxicam (41.44 ± 1.27 μg ml⁻¹ h²) in comparison to cefquinome given (41.89 ± 1.37 μg ml⁻¹ h²) alone (Tiwari et al., 2015) [25]. No significant alteration of AUMC were observed in calves when cefpime co-administered with ketoprofen (338.77 ± 23.35 μg ml⁻¹ h²) in comparison to cefpime given (367.26 ± 36 μg ml⁻¹ h²) alone (Patel et al., 2012) [19]. Patel et al. (2012b) [18] also reported no significant alteration in AUMC in sheep when cepfime coadministered with ketoprofen (1013.18 ± 116.63 μg ml⁻¹ h²) in comparison to cepfime given alone (921.31 ± 84.21 μg ml⁻¹ h²). Similar result regarding AUMC was observed in goats when cepfime coadministered with ketoprofen (999.19 ± 92.37 μg ml⁻¹ h²) in comparison to cepfime given (880.54 ± 121.27 μg ml⁻¹ h²) alone (Patel et al., 2012a). Whereas the AUMC significantly decrease in goats when ceftriaxone co-administered with paracetamol (313.50 ± 6.156 μg ml⁻¹ h²) in comparison to ceftriaxone given (167.14 ± 54.34 μg ml⁻¹ h²) alone (Jimoh et al., 2011) [13].

In present study, there is no significant alteration in mean residence time (MRT) of cefquinome when co-administration with flunixin in goats 2.56 ± 0.07 h in comparison to cefquinome alone. Similarly, no alteration found in the MRT of cefquinome in goats when co-administration with the meloxicam (2.44 ± 0.06 to 2.25 ± 0.05 h) in comparison to cefquinome given alone (Tiwari et al., 2015) supporting present study. Likewise, no significant alteration of MRT were observed in calves when cefpime coadministered with ketoprofen in comparison to cepfime given alone (Patel et al., 2012) [19]. Patel et al. (2012b) [18] also reported no significant alteration in MRT values in sheep when cepfime coadministered with ketoprofen (6.17 ± 0.19 h) in comparison to cepfime given (5.95 ± 0.18) alone. Similar result regarding MRT was observed in goats when cepfime coadministered with ketoprofen (6.70 ± 0.15 h) in comparison to cepfime given (5.97 ± 0.53 h) alone (Patel et al., 2012a) [17]. Similarly no significant alteration of MRT were observed in sheep when cefquinome co-administered with tolfenamic acid (7.27 ± 0.27 h) in comparison to cefquinome given (9.14 ± 1.83 h) alone (Rana et al., 2015) [21].

The apparent volume of distribution (Vdₐₑₐ) of cefquinome coadministered with flunixin in goats was 0.42 ± 0.042 L kg⁻¹ which is significantly higher to the volume of distribution of 0.29 ± 0.02 L kg⁻¹ observed in cefquinome alone. Significant alteration observed in the volume of distribution (Vdₐₑₐ) of ceftriaxone following co-administration of meloxicam in sheep (Ranjan et al., 2011) [22]. However, Jimoh et al. (2011) [13] reported significant decrease in volume of distribution of ceftriaxone co-administered with paracetamol in goats. Whereas no significant alteration found in volume of distribution in goats when cepfime co-administered with ketoprofen (Patel et al., 2012a) [17]. Rana et al. (2015) [21] also found no significant changes in the volume of distribution when cefquinome co-administered with tolfenamic acid (1.48 ± 0.08 L kg⁻¹) in comparison to cefquinome given alone (2.07 ± 0.36 L kg⁻¹).

The apparent clearance (Cl') of cefquinome co-administered with flunixin in goats was 147.41 ± 6.44 ml kg⁻¹ h⁻¹ which is almost similar to 140.33 ± 8.09 ml kg⁻¹ h⁻¹ observed in cefquinome alone.

Conclusion
The finding of the present study showed that no significant alteration in the major pharmacokinetic parameters of cefquinome were observed following its concomitant administration with flunixin in goats so flunixin at the dose rate of 2.2 mg kg⁻¹ body weight intravenously can be successfully co-administered with cefquinome at the dose rate of 2 mg kg⁻¹ intramuscular for combating bacterial infections with an inflammatory condition in goats. Flunixin co-administered with cefquinome in goats, cause no alteration in most of the pharmacokinetics parameters of cefquinome, requiring no revision of dosage regimen. As per general recommendation that T>MIC should be atleast 50% of the dosage interval for optimum bactericidal effect, a 12 h dosing interval at the rate of dose rate 2 mg kg⁻¹ by intramuscular is recommended.

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