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Difference in the PK of ceftiofur in the presence and absence of nimesulide and implications for dose determination through PK/PD integration

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ABSTRACT

The study was planned to determine the pharmacokinetic (PK) and pharmacodynamic (PD) interactions between ceftiofur crystalline free acid (CCFA) and nimesulide (NS). The CCFA was administered to six adult non-lactating goats alone and in combination with NS in a two period cross-over study so that each goat receives each treatment. CCFA (6.6 mg/kg) and NS (2 mg/kg) were injected SC and IM, respectively. Blood samples were collected from jugular vein before and at 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 30, 36, 48, 72, 96, 120, 144, 168, 192, 240, 288 and 336 h post CCFA + NS administration. The MIC, MBC, MPC and *ex-vivo* antibacterial activity of ceftiofur against *P. multocida* were determined in presence of NS. PK-PD data were integrated and modelled to determine AUC_{24h}/MIC values for three levels of effect on the bacteria to predict ceftiofur doses. The MIC, MBC, MPC and *exvivo* growth inhibition curves indicated time-dependent bactericidal activity of ceftiofur against *P. multocida*. The mean peak concentration (C_{max}), absorption (K_{01}HL) and terminal half-lives (K_{10}HL) of ceftiofur were 2.89 µg/mL, 2.31 h and 54.8 h, respectively. The predicted $AUC_{0\text{-}24h}$/MIC ratios required to produce bacteriostatic, bactericidal and eradication activity for *P. multocida* were higher (15.4, 70.1, 114.9 h) with CCFA alone compared to CCFA + NS treatment (12.8 h, 57.6 h, 92.1 h). The study supports using CCFA at 6.6 mg/kg in combination with NS to treat respiratory infectious diseases in goats caused by *P. multocida* since it may decrease the selection for resistant mutants.

1. Introduction

Ceftiofur crystalline-free acid (CCFA) is a sustained-release injectible formulation of ceftiofur that offers an extended duration of action with single injection compared to other short acting antimicrobial formulations. It has been approved for treatment of bovine respiratory disease (BRD, shipping fever, pneumonia), bovine foot rot in beef, non-lactating dairy and lactating dairy cattle; acute metritis caused by bacteria susceptible to ceftiofur in lactating dairy cattle; and respiratory tract infections in swine and equines. The pharmacokinetics (PK) following extra-label use of CCFA have been investigated in alpacas (Dechant et al., 2013), American black ducks (Hope et al., 2012), Asian elephants (Adkesson et al., 2012), Guinea fowl (Wojick et al., 2011), and domestic goats (Dore et al., 2011; Fernández-Varón et al., 2016; Waraich et al., 2016) and differences in PK parameters between species have been reported.

Non-steroid anti-inflammatory drugs (NSAIDs) are often used as an adjunctive therapy with antimicrobial agents in the treatment of respiratory tract infections to reduce the excessive inflammatory response secondary to bacterial and viral infections in veterinary medicine (Lockwood et al., 2003; Brentnall et al., 2012; Salichs et al., 2013; Thiry et al., 2014). Nimesulide (NS, 4-nitro-2-phenoxymethane sulfonanilide) is among the most prescribed COX-2 selective NSAIDs worldwide (Bennet and Villa, 2000; Rainsford, 2006). Nimesulide has potent anti-inflammatory activity and is frequently used in combination with antimicrobials for treatment of diseases like acute mastitis, pneumonia and other bacterial respiratory infections in farm animals in India (Mahapatra et al., 2009). In cows, combination treatment of enrofloxacin with NS was found more efficacious in treating subclinical mastitis compared to enrofloxacin alone (Joshi and Gokhale, 2006).

The therapeutic outcome of antimicrobial agents may be altered (reduced efficacy or increased toxicity) by co-administration with

Abbreviations: BRD, Bovine respiratory disease; CCFA, Ceftiofur crystalline free acid; IM, Intramuscular; MIC, Minimum inhibitory concentration; MBC, Minimum bactericidal concentration; MPC, Mutant Prevention concentration; MHB, Mueller Hinton Broth; NS, Nimesulide; NSAIDs, Non-steroid anti-inflammatory drugs; PK, Pharmacokinetics; PD, Pharmacodynamics; SC, Subcutaneous

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NSAIDs due to PK and PD interactions (Whittem et al., 1996; E.L-Banna, 1999; Varma et al., 2000). Marbofloxacin altered the PK of tolfenamic acid following the concurrent administration of marbofloxacin + tolfenamic acid in calves and goats (Sidhu et al., 2005, 2006).

For antimicrobials, optimizing dosage regimens is an important consideration for achieving clinical success, bacteriological cure and preventing the emergence of resistance. Therefore, it is important to determine the effect of NS on optimum dosage regimen of CCFA before using CCFA off label in combination with NS in goats. In sheep and goats, respiratory tract infections or pneumonia associated with *P.multocida* are frequently encountered in field leading to severe economic losses (Momin et al., 2011; Sirous et al., 2011; Salaheddin and Hanan, 2012). In United States, no NSAID has been approved for use in small ruminant species and NSAIDs are used extra-label in these species. However, in India, NS (Nimovet: Nimesulide injection 10%) is labeled to use in farm animal species including sheep and goats. Ceftiofur and NS may be used together to treat infections in the small ruminant species, but PK-PD interactions of CCFA and NS have not been studied in goats. Therefore, purpose of the study was to evaluate the PK and PD interactions between CCFA and NS and determination of rational dosage regimen of CCFA administered with NS in goats.

2. Materials and methods

2.1. Animals

The study was conducted in accordance to ethical guidelines on the proper care and use of animals set by the Institutional Animal Ethics Committee of Guru Angad Dev Veterinary and Animal Sciences University, India with reference number GADVASU/2013/IAEC/18/LA006. Six Beetal non-lactating female goats between 6 months to one year of age and 35–50 kg body weight were housed individually in a ventilated barn, fed on green fodder and had unrestricted access to water.

2.2. Experimental design

A two-period cross over design (3 × 3) with two treatments was used, such that randomly each goat received each treatment of CCFA (EXCEDE; Zoetis Inc., Madison, New Jersey-07940, USA) and CCFA + NS (Nimovet 10% injectable; Immunologicals Ltd. Group, Hyderabad, India). Ceftiofur standard was purchased from Sigma Aldrich, India. The CCFA was injected at a dose rate of 6.6 mg/kg body weight subcutaneously (SC) on the left side of the neck region of goats and NS was administered intramuscularly (IM) over the right gluteal muscle at a dose of 2 mg/kg body weight at the same time. Blood samples (10 mL) were collected from lateral jugular vein at time intervals *viz*. 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 30, 36, 48, 72, 96, 120, 144, 168, 192, 240, 288 and 336 h post CCFA + NS administration. Serum was separated from blood samples by centrifugation at 2000*g* for 15 min and stored at −20 °C till further analysis. Blood samples were also collected for establishing *ex-vivo* antibacterial activity of CCFA in presence of NS against *P. multocida* at predetermined times and serum was frozen at −20 °C.

2.3. Analysis of samples for ceftiofur concentration

The concentration of ceftiofur in serum were measured as ceftiofur and its metabolite, desfuryl-ceftiofur (DFC) on an automated HPLC-UV system (Perkin Elmer Series 200) using a validated method modified from others (Baere et al., 2004; Jacobson et al., 2006). A 500 µL of serum samples and standards were derivatized to DFC acetamide followed by solid-phase extraction as described elsewhere in our companion paper (Waraich et al., 2016). The flow rate of mobile phase was set at 1 mL/min. The retention time of the ceftiofur + DFC was 22.5–23.5 min with a total run time of 25 min and equilibration time of 2 min. The UV detector was set at a wavelength of 265 nm. Calibration curve equations were obtained by fitting peak area ratios versus standard ceftiofur concentration using a least square linear regression. The calibration curve for the assay was linear over the concentration range of 0.05–10 µg/mL in goat serum, regression coefficient, $r^2 = 0.99$. The lower limit of quantification (LLOQ) and lower limit of detection (LLOD) of the analytical method were 0.10 µg/mL and 0.05 µg/mL, respectively. Intra- and inter-day assay precision and accuracy levels were < 5% and > 95%, respectively.

2.4. Pharmacokinetic data analysis

For each goat, the ceftiofur serum concentration data following CCFA + NS administration were fitted to a compartmental PK model to estimate parameter values for ceftiofur using nonlinear regression analysis as implemented in the commercially available computer software program; [e] Phoenix® Win-Nonlin, Certara USA Inc. Princeton, NJ, USA (Gibaldi and Perrier, 1982). The data were fitted to one-compartment model with first order absorption and elimination and best fit was based on Akaike's information criterion, Minimum Akaike Information Criterion Estimation (MAICE) test as well as the visual inspection of the fitted curves.Using parameters (V/F, K_{10}, K_{01}) obtained by curve fitting, CL and K_{10} HL were calculated as $CL/F = V/F^*K_{10}$ and K_{10} HL = $0.693/K_{10}$. Non-compartment analysis based on the statistical moment theory was used to determine area under the first moment curve (AUMC) and mean residence time (MRT) (Perrier and Mayersohn, 1982).

2.5. Determination of MIC, MBC and MPC

A quality control (QC) strain of *P. multocida* (B:2 P52) was obtained from Punjab Veterinary Vaccine Institute, Ludhiana, India and field strains of *P. multocida* was isolated from 18 goats clinically suffering from pneumonia.To study the effect of nimesulide on *P. multocida* susceptibility to ceftiofur, the minimum inhibitory concentration (MIC), minimum bactericidal concentrations (MBC) and mutant prevention concentration (MPC) of ceftiofur were determined in the absence and presence of nimesulide. For the latter, goat serum and Muller Hinton Broth (MHB) were added with nimesulide (1 µg/mL) at the time of the start of the experiments. The MIC of ceftiofur was determined in MHB and goat serum using micro-dilution method with *P. multocida* inoculum of $4–6 \times 10^7$ CFU/mL (Blondeau et al., 2001; Sidhu et al., 2010; CLSI, 2013). Minimum bactericidal concentration (MBC) was determined using sheep blood agar plates (bacterial count reduction by 1000 fold of initial inoculum).

The ceftiofur MPC was determined using a standardized method initially described by Blondeau and co-workers (Blondeau et al., 2001; Balaje et al., 2013). For each experiment a bacterial density of *P. multocida* ≥ 10^{10} CFU/mL was used. Blood agar plates containing known concentrations of ceftiofur (multiples of MIC) were inoculated with 100 µL of this culture and incubated at 37 °C for 72 h. Plates were checked for growth of *P. multocida* every 24 h after incubation. The MPC was recorded as the lowest multiple of MIC that completely inhibited bacterial growth for 72 h.

2.6. Ex-vivo antibacterial activity of ceftiofur

For each time point, serum samples from six goats were pooled to establish *ex vivo* antibacterial activity of ceftiofur because results from individual animals were same in first period study (Waraich et al., 2016). *Ex-vivo* antibacterial activity of ceftiofur against *P. multocida* in MHB and serum was determined using a micro-dilution method at selected time points after administration of CCFA + NS (Sidhu et al., 2010; Balaje et al., 2013). The serum samples (400 µL) were inoculated with 10 µL of stationary phase bacterial culture in MHB to give a final