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Disposition Kinetics of Ceftriaxone and Sulbactam (1:1) in Black Bengal Goats with Experimental Mastitis

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ABSTRACT

The pharmacokinetics of ceftriaxone, sulbactam individually and in combination (ceftriaxone: sulbactam, 1:1) at 20 mg kg^{-1} body weight (bw) as single dose intravenous administration (i.v.) was conducted in healthy and experimental Escherichia coli O157:H7 induced mastitis in black Bengal goats. Semi logarithmic plot of plasma ceftriaxone and sulbactam concentrations against time in healthy and mastitis induced goats alone and in combination witnessed 2-compartment models. Ceftriaxone could not be detected in plasma beyond 6 h in individual therapy and 8 h in combination therapy with sulbactam. Ceftriaxone persisted in the milk for longer time in experimental mastitis when given in combination with sulbactam. The lower $C_{max\ milk}$: $C_{max\ plasma}$ (≤1) values indicated inadequate distribution and penetration of ceftriaxone into the mammary glands in experimental mastitis. Ceftriaxone was rapidly metabolized to ceftizoxime post-dosing which was also detected in the plasma and milk for considerable period of time. Mastitis-challenged goats treated with sulbactam had grave prognosis. Pharmacokinetics of both ceftriaxone and sulbactam got altered in goats post experimental mastitis. The dosage of the drugs in combination therapy of ceftriaxone with sulbactam may be reduced in experimental mastitis compared to healthy goats.

Keywords: Ceftriaxone, sulbactam, ceftizoxime, Escherichia coli mastitis, pharmacokinetics.

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1. INTRODUCTION

Clinical mastitis is recognized worldwide to cause major economic losses in dairy cattle because of the loss in milk production and quality (De Graves & Fetrow, 1993; Watts *et al*., 1995). Among which, *Escherichia coli* is among the most common infectious agent isolated from severe mastitis cases in modern dairy farms (Hogan et al., 1989; Bradley et al., 2007).

Broad-spectrum antimicrobials are commonly used systemically or as an intramammary in the treatment of acute E. coli mastitis (Erskine et al., 2003). However, results of the treatment studies have been controversial. No difference was established between groups treated with or without antimicrobials in experimentally induced E. coli mastitis (Erskine et al., 1992; Pyorala et al., 1994), while other studies reported some benefits of antimicrobial treatment (Shpigel et al., 1997; Rantala et al., 2002; Poutrel et al., 2008). Parenteral administration of broad-spectrum antimicrobials has been recommended for the treatment of severe coliform mastitis, due to the risk of bacteremia (Cebra et al., 1996; Wenz et al., 2001b). As the intra-mammary infections in dairy goats are mainly bacterial origin and E. coli is one of the most prevalent one in dairies (Shpigel *et al.* 1997; Martin *et al*. 2007; Shathele, 2009), it demands a study on alternative antimicrobials, specifically for small ruminants as they also contribute to the dairy milk production in developing countries.

Every attempt should be made in developing a consensus about efficient, safe and economical treatment of coliform mastitis in small ruminants. While considering cephalosporins for the same, the capability of ceftriaxone is well established in the treatment of septicemia, bacteremia, lower respiratory tract infection, urinary tract infection, peritonitis, enteritis and soft tissue infections due to its potential to penetrate the extra-vascular space in human beings (Patel *et al.* 1984; Tan *et al.* 1984). Therefore, more studies must be conducted to explore its disposition kinetics, which may reflect its efficacy in the treatment of small ruminant mastitis as an alternative therapy in the absence of other antimicrobial treatment options. However, some experiments suggest that, kinetic profile of the ceftriaxone varies with animal species, disease conditions and route of administration (Sar *et. al.* 2006; Goudah, 2008). Besides, ceftriaxone metabolite ceftizoxime was also found in high concentrations in milk post administration (Sar *et al.*, 2006). Hence, there is a chance that presence of sulbactam and metabolite ceftizoxime can alter the bioavailability of the ceftriaxone in the goat body system including milk. At present, there is scarcity among data to support the hypothesis drawn here. Therefore, the objective of the study was to compare the absorption, distribution, metabolism and elimination rates of ceftriaxone and sulbactam in goats to design a drug-dosage regimen and to determine milk penetration, following *i.v.* administration of a single dose (20 mg/kg each) ceftriaxone/sulbactam (1:1) combination in both healthy and experimental mastitis conditions.

2. MATERIALS AND METHODS

Chemicals

Most of the pharmaceutical ceftriaxone-sulbactam combination available currently is in the ratio of 2:1. Hence, technical grade (purity 98.5%, Alembic Private Limited, Bombay, India) of Ceftriaxone, ceftizoxime and sulbactam was used for the study to achieve a desired ratio 1:1 of parent drugs. All other chemicals used in this study were obtained from E. Merck (Mumbai, India) except heparin which was from Sigma Chemical Co. (St Louis, MO, USA).

Animal Treatment

Thirty clinically healthy lactating adult female black Bengal goats weighing between 8-12 kg of approximately 1½ - 2 year age were used. At least thirty days prior to the onset of the study all the goats were dewormed with a single oral dose of 10 mg Kg^{-1} Albendazole, stabled and fed with an antibiotic free diet with *ad libitum* water. Approval of the study plan from the concerned animal ethics committee was obtained.

Isolation of Escherichia coli

Milk samples from clinical cases were screened to identify the responsible coliforms for clinical mastitis in cows and goats. The samples were collected aseptically as recommended by Clinical and Laboratory Standards Institute (2007). The samples were transported to the laboratory at 4^0C within 24 hours of collection. The isolation and identification of *Escherichia coli* O157:H7 was performed using standard bacteriological methodology suggested by Clinical and Laboratory Standards Institute (2007). As most of the coliforms isolated from the different dairy animals including goats were positive for *Escherichia coli* O157:H7, therefore, it was decided to standardize the same for the induction of mastitis to carry out pharmacokinetic studies.

Induction of experimental mastitis

Intra-mammary infections in dairy goats are mainly bacterial in origin (Martin et al., 2007) and we found that coliforms were one of the causative agents in isolated mastitis samples from the clinical cases of the goats. Therefore, to gain more knowledge about small ruminant coliform mastitis, slant culture of *Escherichia coli* O157:H7obtained from the above source was made in Muller Hinton broth (*MHB*) and kept for 24 h in incubator at 37^0C. Immediately after that the

subculture was made in *MHB* broth and incubated at 37^0C for 18 h. This subculture was preserved at 4^0C after serial dilution. Taking 1 mL of this broth subculture, serial dilution was performed in 9 sugar tubes containing 9 mL of *MHB* each and pH 7.2 under strict aseptic condition. One mL of broth subculture from sugar tubes having dilution of 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} were poured and spread on plate containing tryptose agar. These 5 plates were incubated at 37^0C for 24 h and the colony numbers were counted. Plate having broth culture of 10^{-6} dilution, showed 40 colony forming unit (*CFU*) was used for induction of mastitis. The serial dilution process was again repeated by taking the original broth culture preserved at 4^0C up to 10^{-6} dilution.1 mL of this diluted subculture was administered *via* teat canal to each of the two quarters to a group of five goats of for experimental induction of mastitis. California mastitis test, bromothymol blue paper test, somatic cell count (IDF, 1995) and milk lactoperoxidase activity (Makinen and Tenovuo, 1982) of the respective goats were used for diagnosis of mastitis and were recorded daily to keep in check the severity of mastitis.

The minimum inhibitory concentration (*MIC*) is defined as the lowest antimicrobial concentration that is capable of preventing the assessed microorganism from growing (Garcı´a Rodrı´guez et al., 2000). On exposure of *Escherichia coli O157:H7* MIC to minimum bactericidal concentration (*MBC*), sulbactam had very high MIC of 64 µg mL^{-1} and MBC of 128 µg mL^{-1} in tested inoculums. Ceftriaxone demonstrated substantial inoculum effect with its *MIC* and *MBC* of 1 and 2 µg mL^{-1}, respectively. The combination of sulbactam and ceftriaxone expressed maximum inoculum effect with *MIC* and *MBC* of 0.39 and 0.78 µg mL^{-1}, respectively. Therefore, these finding suggests that the ceftriaxone sulbactam combination therapy has a potential of treating goat coliform mastitis and prevent systemic infections, if we are able to maintain a MIC in blood and milk with an appropriate ceftriaxone: sulbactam dosage regimen.

Study Design

For the pharmacokinetic study, a total of 30 clinically healthy lactating black Bengal goats were divided into six groups having five goats each (*viz.* Healthy Group I CTX, Healthy Group II SUL, Healthy Group III CTX:SUL, Mastitis Group I CTX, Mastitis Group II SUL and Mastitis Group III CTX:SUL). Then the experiment was divided into two parts (i) Pharmacokinetic study of ceftriaxone, sulbactam and combination (ceftriaxone: sulbactam, 1:1) at 20 mg kg^{-1} bw as single dose i.v. in Healthy Group I CTX, Healthy Group II SUL and Healthy Group III CTX:SUL, respectively. (ii) Pharmacokinetic study of ceftriaxone, sulbactam and combination (ceftriaxone: sulbactam, 1:1) at 20 mg kg^{-1} BW as single dose i.v. in experimental Mastitis Group I CTX, Mastitis Group II SUL and Mastitis Group III CTX:SUL, respectively. Blood samples for analysis of ceftriaxone, ceftizoxime and sulbactam were collected at 0 (control), 0.08, 0.16, 0.33, 0.66, 1, 2, 4, 6, 8, 12, 24 and 48 hours after drug administration. Plasma was then separated by centrifugations at 1500 *g* for 20 minutes. Milk samples were also collected from both teats separately into the test tubes at 0, 0.16, 0.50, 1, 2, 6, 12, 24, 48, 72, 96, 120, 144 and 168 hours post dosing. Milk and plasma samples were stored at -20^0 C.

Equilibrium dialysis technique, as described by Sisodia *et al.* (1965), Banerjee *et al.* (1969), modified by Mandal *et al*. (1990) and Dow (2006) was used to determine plasma protein binding (*P.P.B.*) and milk protein binding (*M.P.B.*) of ceftriaxone and sulbactam. The plasma and milk pH and protein concentrations of freshly collected, pooled drug-free plasma and milk from five goats were determined prior to the assay. Analytical grade of ceftriaxone was added to 1 mL plasma aliquots to yield concentrations of 0.67, 6.67 and 66.67 µg/mL simultaneously, sulbactam samples with same concentrations were prepared. Same procedure was repeated for milk samples. Samples were incubated at 104 °F to replicate goat core body temperature for 30 minutes and were centrifuged in a fixed angle rotor centrifuge at 4000 g for 30 min, using an ultrafiltration device (Amicon Ultra-0.5 30 kDa; Merck Millipore, Kilsyth, Victoria, Australia). The non protein bound ultra filtrate (Drug unbound) was recovered and the drug concentrations were determined. This was compared with the initial concentration added prior to ultra filtration (Drug total). Samples were prepared and analyzed in triplicate. Percentage binding to plasma and milk proteins was determined using the following equation:

$$\% \text{ Protein binding} = \frac{\text{Drug total} - \text{Drug unbound}}{\text{Drug total}} \times 100$$

The quantity of metabolite ceftizoxime recovered was correlated with the parent compound by effective ratio (ER= molecular weight of metabolite/ molecular weight of parent), following which the quantity of parent compound (P= quantity of metabolite recovered/ ER) which produce same metabolite expressed in µg mL^{-1} was calculated and pharmacokinetic parameters were analyzed. Ceftriaxone and sulbactam dosage regimen was designed using standard equations by Rowland and Tozer, (1995).

Analytical method

Plasma and milk concentrations of ceftriaxone, ceftizoxime and Sulbactam were analyzed by SHIMADZU LC-20 AT liquid chromatography coupled with Photo Diode-Array detector according to the modified method of Blum *et al.,* (1989), Sar *et al.*, (2006) with the extraction phase described by Rudrik and Bawdon (1981). For sulbactam, ceftizoxime and ceftriaxone in the extraction phase, to 0.5 mL of milk or plasma was added with 0.5 mL of mobile phase ($_P$H=6.1). The samples were mixed and was added with 3 mL of acetonitrile (HPLC grade) centrifuged at 2000 *g* in Remi R8C centrifuge for 10 minutes. The supernatant decanted into 3 mL of dichloromethane (DCM) in a screw cap tube to separate the aqueous layer by centrifuging at 3000 *g* for 10 minutes. Samples (20µl) of upper aqueous layer were injected into a column (5 µ Luna C 18 (2); 250 × 4.6 mm RP) *via* precolumn of HPLC with following specifications; and were scanned by an ultraviolet detector at 254 nm and 0.02 absorbance units full for ceftriaxone, ceftizoxime and at 313 nm, 0.02 absorbance units full for sulbactam . For ceftriaxone and ceftizoxime the mobile phase was 80% phosphate buffer (0.1M, pH=6.1) and 20 % acetonitrile. This mixture was subjected to membrane filtration (0.45µ pore size). The flow rate was 1 mL min^{-1} and retention time of ceftizoxime and ceftriaxone was 3.584 min and 4.931 min respectively. For sulbactam, the mobile phase was 12% acetonitrile plus 88% phosphate buffer (0.1M; pH=6.1). 4 mL of tetrabutylammonium hydroxide (20% solution in water) was added per litre of mobile phase; the flow rate was adjusted to 2 mL/min which resulted in the retention time of 6.56 minutes. In the Sulbactam assay, the plasma samples (1 mL) were previously derivatized (Bawdon & Madsen, 1986) using imidazole reagent (8.5 g of imidazole reagent in 20 mL of distilled water, with the addition 5 N hydrochloric acid to bring the solution to $_p$H of 6.8 and the volume adjusted to 40 mL with water). The method was validated by measuring known concentrations of ceftriaxone, ceftizoxime and sulbactam in plasma and milk.

Pharmacokinetics and Statistical analysis

Pharmacokinetic parameters of ceftriaxone and sulbactam were determined from computerized curve fitting program 'PHARMKIT' by the Department of Pharmacology, JIPMER, Pondicherry, India. Pharmacokinetic parameters were determined for each animal individually calculating the mean and standard error (SE). Mean values, SE and analysis of variance of the tabulated data were calculated where applicable by using standard formulas.

3. RESULTS:

Drug analysis results suggested that the average (Mean ± Standard error) recoveries between and within batch for ceftriaxone was 94.01±0.23 and 95.23±0.32%, for ceftizoxime 96.01±0.63 and 97.11±0.21% and for sulbactam were 92.01±0.55 and 93.23±0.12%, respectively. Assayed values varied less than ±4% from calculated values. The assay was linear from 0.05 to 100 µg mL^{-1} for all the drugs. The limits of quantification were 0.05 µg mL^{-1} for ceftriaxone, 0.04 µg $mL^{-1}$for ceftizoxime and 0.06 µg mL^{-1} for sulbactam.

The mean plasma concentrations against time of ceftriaxone, metabolite ceftizoxime in experimental coliform mastitis and healthy goats are plotted in **Figure 1** and sulbactam in **Figure 2**. Pharmacokinetic parameters are summarized in **Table 1 and 2**. Ceftriaxone and sulbactam concentrations in plasma decreased in a bi-exponential manner after i.v. administration, indicating the presence of distribution and elimination phases and justifying the use of a two-compartment kinetic model for analyzing the data. The apparent volume of distribution (Vd_{area}) of ceftriaxone when given in combination with sulbactam was significantly higher 1.12±0.08 L Kg^{-1} in healthy goats compared to the goats having experimental mastitis, so was the elimination half life ($t_{1/2}\beta$) value of 3.48±0.11 h. However, after the induction of experimental coliform mastitis and exposing the goats to ceftriaxone: sulbactam combination, significantly higher values of $C^0{}_P$, C_{max} and *AUC*, of 240.76±16.22 µg mL^{-1}, 115.46±12.0 µg mL^{-1} and 162.04±16.14 µg h mL^{-1}, respectively, were obtained. *T~P* ratio (3.36) of ceftriaxone was also higher in combination therapy in experimental mastitis as compared to the healthy goat individual treatment ratio (2.09). Ceftriaxone was metabolized to ceftizoxime, both in individual and combination therapy however, on experimental mastitis ceftizoxime was detected for longer duration of up to 8 hours. Post experimental mastitis, the plasma sulbactam level (262.03±9.78 µg mL^{-1}) was significantly higher in combination therapy with ceftriaxone. Also, sulbactam was transferred relatively faster from the central compartment to the peripheral compartment (11.48^b±0.73 h^{-1}) in combination with ceftriaxone in experimental mastitis. Also, significantly higher total body clearance (Cl_B) of 0.22±0.01L kg^{-1} h^{-1} was attained in healthy goats with ceftriaxone: sulbactam combination. Both ceftriaxone and sulbactam followed similar kinetic patterns.

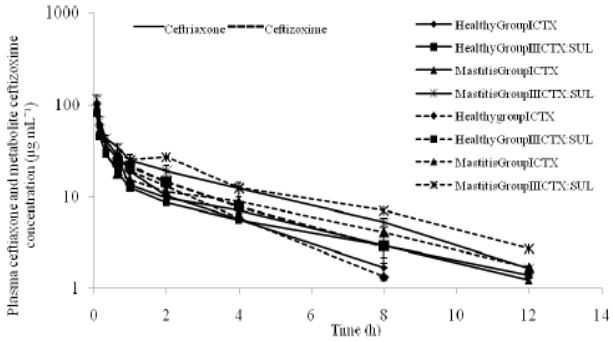

Figure 1: Mean plasma concentrations of ceftriaxone and its metabolite ceftizoxime after single *i.v.* at 20 mg kg^{-1} *bw* alone and in combination (ceftriaxone: Sulbactam, 1:1) to healthy and experimental mastitis of black Bengal goats†.

Groups; Healthy Group I CTX- ceftriaxone alone in healthy goats, Healthy Group II SUL- sulbactam alone in healthy goats, Healthy Group III CTX: SUL- ceftriaxone in combination with sulbactam in healthy goats, Mastitis Group I CTX- ceftriaxone alone in goats with experimental mastitis, Mastitis Group II SUL- sulbactam alone in mastitis in goats with experimental mastitis, Mastitis Group III CTX: SUL- ceftriaxone in combination with sulbactam in goats with experimental mastitis

†Mean with standard error of 5 replicates

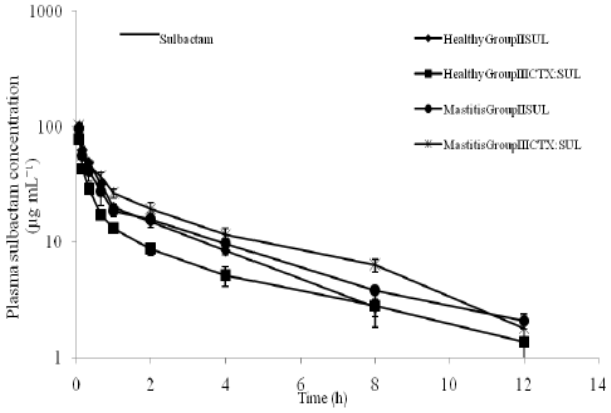

Figure 2: Mean plasma concentration of sulbactam after single i.v. at 20 mg kg^{-1} *bw* alone and in combination (Ceftriaxone: Sulbactam, 1:1) in healthy and experimental mastitis of black Bengal goats†

Kinetic parameters	Ceftriaxone				Metabolite ceftizoxime (CFZ)			
	Healthy Group I CTX	Healthy Group III CTX:SUL	Mastitis Group I CTX	Mastitis Group III CTX:SUL	Healthy Group I CTX	Healthy Group III CTX:SUL	Mastitis Group I CTX	Mastitis Group III CTX:SUL
Plasma								
C^0_P(µg mL^{-1})	181.38^{b}±20.66	95.55^{a}±9.83	123.77^{a}±3.49	240.76^{c}±16.22	–	–	–	–
C_{max} (µg mL^{-1})	101.43^{ab}±5.11	81.57^{a}±7.13	95.54^{ab}±4.57	115.46^{b}±12.0	31.3^{a}±3.34	34.44^{ab}±3.02	25.97^{a}±2.57	41.65^{b}±2.85
β(h^{-1})	0.36^{c}±0.03	0.19^{ab}±0.01	0.22^{a}±0.01	0.25^{b}±0.01	0.53^{c}±0.01	0.40^{b}±0.01	0.28^{a}±0.01	0.27^{a}±0.01
$t_{1/2}\beta$(h)	1.82^{a}±0.13	3.48^{c}±0.11	3.01^{b}±0.13	2.68^{b}±0.12	2.60^{a}±0.11	3.50^{a}±0.10	5.02^{b}±0.43	5.2^{b}±0.39
AUC(µg h mL^{-1})	94.69^{a}±4.23	88.75^{a}±4.19	98.02^{a}±2.69	162.04^{b}±16.14	110.26^{a}±8.8	141.01^{a}±6.52	1401.1^{a}±10.23	238.95^{b}±0.97
Cl_B(L kg^{-1} h^{-1})	0.23^{b}±0.01	0.21^{b}±0.01	0.19^{b}±0.01	0.12^{a}±0.01	0.03^{c}±0.01	0.02^{b}±.001	0.02^{b}±0.01	0.01^{a}±0.001
Vd_{area}(L Kg^{-1})	0.57^{a}±0.03	1.12^{c}±0.08	0.87^{b}±0.06	0.46^{a}±0.04	0.10^{a}±0.01	0.10^{a}±0.01	0.15^{b}±0.013	0.08^{a}±0.01
K_{el}(h^{-1})	1.95^{b}±0.23	1.09^{a}±0.07	1.22^{a}±0.06	1.50^{ab}±0.15	–	–	–	–
K_{12}(h^{-1})	6.96^{b}±0.85	3.27^{a}±0.55	3.65^{a}±0.39	8.67^{b}±0.72	–	–	–	–
K_{21}(h^{-1})	2.05^{b}±0.13	0.90^{a}±0.06	0.99^{a}±0.01	2.14±0.40	–	–	–	–
F_C	0.19^{a}±0.01	0.17^{a}±0.01	0.18^{a}±0.01	0.17^{a}±0.02	–	–	–	–
MRT(h)	4.11^{a}±0.30	4.57^{a}±0.23	4.44^{a}±0.52	4.90^{a}±0.77	3.63^{a}±0.08	5.81^{b}±0.82	7.05^{b}±0.66	7.33^{b}±0.56
T~P	2.09^{a}±0.13	4.07^{b}±0.20	3.49^{b}±0.14	3.36^{b}±0.14	–	–	–	–

Table 1: continued

Milk								
$C_{max}(\mu g\ mL^{-1})$	13.64^{b}±2.02	9.15^{ab}±0.87	8.49^{a}±0.24	9.1^{ab}±1.50	46.13^{a}±6.29	46.31^{a}±2.97	34.81^{a}±1.78	27.02^{a}±0.36
$t_{1/2}\beta(h)$	14.98^{b}±1.52	4.68^{a}±1.02	13.77^{b}±0.24	14.30^{b}±1.31	29.86^{b}±0.78	45.09^{b}±5.01	32.05^{a}±4.75	37.95^{ab}±1.94
$Vd_{area}(L\ Kg^{-1})$	0.96^{b}±0.08	0.33^{a}±0.03	1.67^{c}±0.09	0.97^{b}±0.122	0.14^{a}±0.01	0.36^{b}±0.07	0.42^{b}±0.07	0.29^{b}±0.01
AUC $(\mu g\ h\ mL^{-1})$	459.58^{b}±81.22	858.86^{c}±71.0	238.09^{a}±12.26	428.08^{b}±29.78	8102.9^{b}±67.0	5349.2^{a}±523.05	3830.43^{a}±207.7	3873.63^{a}±379.5
$MRT\ (h)$	23.18^{ab}±2.13	19.85^{a}±0.58	22.03^{ab}±0.42	26.45^{b}±1.47	64.62^{a}±0.73	78.40^{c}±4.13	66.13^{ab}±1.31	74.5^{bc}±8.98
Cl_B $(L\ kg^{-1}\ h^{-1})$	0.04^{b}±0.008	0.021^{a}±0.01	0.08^{c}±0.01	0.04^{b}±0.003	–	–	–	–
AUC_{milk}/AUC_{plasma}	4.85^{b}±0.63	9.6^{c}±1.56	2.42^{a}±0.32	2.64^{a}±0.23	106.63^{c}±9.1	54.98^{a}±5.40	39.49^{a}±3.26	24.18^{b}±2.86
$C_{max\ milk}/C_{max\ plasma}$	0.13^{b}±0.04	0.11^{c}±0.01	0.08^{a}±0.02	0.07^{a}±0.01	2.13^{c}±0.27	1.94^{a}±0.33	1.94^{a}±0.02	0.92^{b}±0.13

Table 1: Mean plasma and milk concentrations of ceftriaxone, its metabolite ceftizoxime following single dose intravenous administration at 20 mg kg^{-1} *bw* alone and in combination (Ceftriaxone: Sulbactam, 1:1) in healthy and experimental mastitis of black Bengal goats†‡•.

‡Parameters; Mean with the similar superscripts within a row do not vary significantly ($p<0.05$); $C^{0}_{P,}$ zero time plasma concentration; $C_{max,}$ peak drug concentration; β, rate constant related to slope of elimination curve; $t_{1/2}\beta$, elimination half life; *AUC*, total area under the curve of plasma drug concentration *versus* time from '*0*' to '*tα*' after administration of a single intravenous dose; $Cl_{B,}$ total body clearance; Vd_{area}, Apparent volume of distribution of drugs on the total area under curve of plasma drug concentration *versus* time; K_{el}, first order rate constant for drug elimination from the central compartment; $K_{12,}$ first order rate constant for transfer of drug from peripheral compartment; $K_{21,}$ first order rate constant for transfer of drug from peripheral to central compartment; $K_{21,}$ first order rate constant for transfer of drug from central to peripheral compartment; $F_{C,}$ fraction of the amount of the drug in the central compartment; *MRT,* mean residential time of the drug in the central compartment; *T~P,* Tissue plasma ratio of the drug;

• Mean with the similar superscripts within a row do not vary significantly ($p<0.05$);

Kinetic parameters	Sulbactam			
	Healthy Group I SUL	Healthy Group III CTX:SUL	Mastitis Group I SUL	Mastitis Group III CTX:SUL
Plasma				
$C^{0}_{P}(\mu g\ mL^{-1})$	130.14^{a}±11.69	101.09^{a}±10.33	122.53^{a}±9.03	262.03^{b}±9.78
$\beta(h^{-1})$	0.31^{b}±0.03	0.20^{a}±0.01	0.19^{a}±0.03	0.25^{ab}±0.01
$t_{1/2}\beta(h)$	2.22^{a}±0.22	3.33^{ab}±0.14	3.75^{b}±0.65	2.72^{ab}±0.06
$AUC(\mu g\ h\ mL^{-1})$	118.82^{ab}±6.48	86.44^{a}±3.62	139.13^{bc}±10.11	170.89^{c}±16.18
$Cl_B(L\ kg^{-1}\ h^{-1})$	0.15^{a}±0.01	0.22^{b}±0.01	0.11^{a}±0.02	0.11^{a}±0.01
$Vd_{area}(L\ Kg^{-1})$	0.52^{ab}±0.05	1.07^{c}±0.06	0.65^{b}±0.04	0.47^{a}±0.03
$K_{el}(h^{-1})$	1.06^{a}±0.12	1.13^{ab}±0.11	0.74^{a}±0.08	1.57^{b}±0.20
$K_{12}(h^{-1})$	3.36^{a}±0.61	3.66^{a}±0.39	3.35^{a}±0.88	11.48^{b}±0.73
$K_{21}(h^{-1})$	1.70^{b}±0.27	1.01^{a}±0.01	1.27^{ab}±0.08	2.46^{c}±0.14
F_C	0.28^{b}±0.01	0.18^{a}±0.01	0.25^{b}±0.03	0.16^{a}±0.01
$MRT(h)$	2.40^{a}±0.05	4.49^{bc}±0.41	3.04^{ab}±0.50	5.26^{c}±0.66
$T\sim P$	2.76^{a}±0.28	3.93^{b}±0.23	4.25^{b}±0.39	3.47^{b}±0.13

Table 2: continued

Milk				
$C_{max}(\mu g\ mL^{-1})$	7.85^{b}±1.05	10.89^{c}±0.36	5.20^{a}±0.22	6.81^{ab}±0.81
$t_{1/2}\beta(h)$	5.81^{a}±0.602	11.84^{b}±1.51	4.51^{b}±0.462	15.53^{a}±1.81
$Vd_{area}(L\ Kg^{-1})$	0.26^{a}±0.05	0.49^{b}±0.06	0.22^{b}±0.05	0.93^{a}±0.07
$AUC(\mu g\ h\ mL^{-1})$	658.55^{ab}±82.79	691.60^{b}±11.33	611.03^{b}±79.62	475.19^{b}±32.15
$MRT(h)$	14.40^{a}±0.91	21.68^{b}±2.46	13.10^{a}±0.90	26.2^{b}±1.30
$Cl_B\ (L\ kg^{-1}\ h^{-1})$	0.03^{a}±0.003	0.11^{a}±0.08	0.03^{a}±0.005	0.03^{b}±0.003
AUC_{milk}/AUC_{plasma}	5.54^{b}±0.96	8.01^{c}±1.78	4.39^{a}±0.56	2.78^{a}±0.72
$C_{max\ milk}/C_{max\ plasma}$	0.07^{a}±.01	0.13^{b}±0.02	0.05^{a}±0.01	0.06^{a}±0.01

Table 2: Mean plasma and milk concentrations of sulbactam following single dose intravenous administration at 20 mg kg^{-1} *bw* alone and in combination (Ceftriaxone: Sulbactam, 1:1) in healthy and experimental mastitis of black Bengal goats†‡•.

Mean milk concentrations against time of ceftriaxone, metabolite ceftizoxime and sulbactam are in **Figure 3 and 4**, while milk pharmacokinetic parameters are in **Table 1 and 2**. Ceftriaxone was discovered in milk after 2 h trough 48 h after administration in all the groups. Ceftriax one was not detected in milk beyond 48 hours in any of the experimental groups. The elimination half life ($t_{1/2}\beta$) of ceftriaxone in combination therapy was very high with 4.62±1.02 h in healthy goats as compared to other respective groups which all took more than10 h for the same. Similarly, the *AUC* of ceftriaxone was significantly higher in healthy goats exposed to combination therapy of ceftriaxone and sulbactam. The AUC_{milk}: AUC_{plasma} ratio of ceftriaxone in milk of healthy goats was almost twice as compared to the goats exposed to experimental mastitis. The same drug had statistically lower $C_{max\ milk}$:$C_{max\ plasma}$ ratio in experimental mastitis as compared to healthy goats. Milk *MRT* of ceftriaxone during experimental mastitis was significantly higher on combination therapy with 26.45±1.47 h. Sulbactam $t_{1/2}\beta$ data suggests that, it has twice the elimination half life in combination therapy as compared to the individual therapy in either of the groups. Also, AUC_{milk}: AUC_{plasma} of sulbactam in combination therapy of healthy goat was significantly higher as compared to experimental mastitis in black Bengal goats.

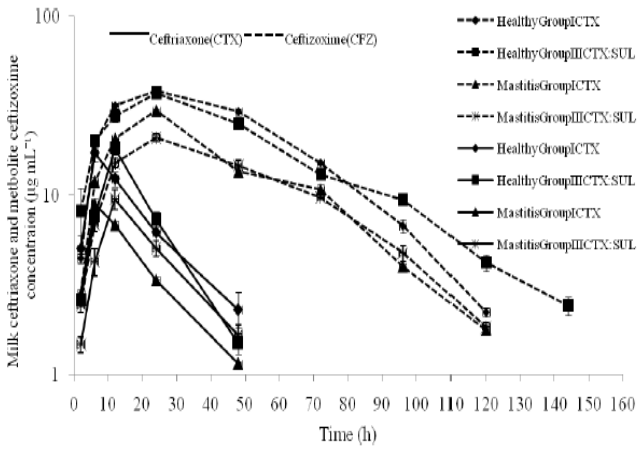

Figure 3: Mean milk concentrations of ceftriaxone and its metabolite ceftizoxime after single dose *i.v* at 20 mg kg^{-1} *bw* alone and in combination (ceftriaxone: Sulbactam, 1:1) to healthy and experimental mastitis of black Bengal goats†.

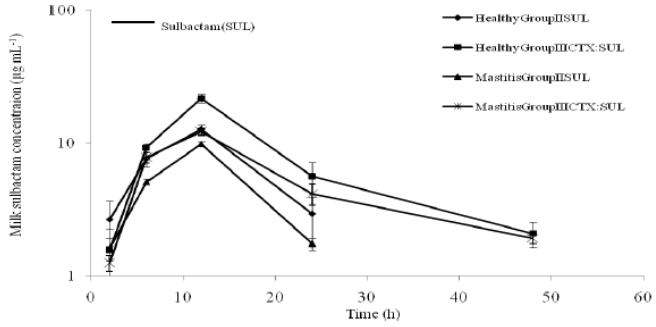

Figure 4: Mean milk concentrations of sulbactam single dose *i.v.* at 20 mg kg^{-1} *bw* alone and in combination (Ceftriaxone: Sulbactam, 1:1) in healthy and experimental mastitis of black Bengal goats**†.**

4. DISCUSSION:

Semi logarithmic plot of plasma ceftriaxone concentrations against time in healthy and mastitis induced goats alone and in presence of sulbactam fitted best to a 2-compartment model. Ismail, (2005) also reported that ceftriaxone serum concentration–time curve expressed a characteristic 2-compartment open model in goats after intravenous administration. Ceftriaxone is eliminated rapidly in healthy goats when administrated individually than in combination with sulbactam, but in experimental mastitis no significant

difference existed between individual and combination therapy. Ismail, (2005) observed that the distribution and elimination half-lives ($t_{1/2\alpha}$, $t_{1/2\beta}$) were 0.12 and 1.44 h respectively in goats. Goudah *et al.,* (2006) also reported that after intravenous dosing, the elimination rate constant and elimination half-life were 0.4 ± 0.05/h and 1.75 ± 0.02 h, respectively in ewes. The significantly higher *AUC* values of ceftriaxone in presence of sulbactam in mastitis induced goats suggest sustained serum concentrations of the parent drug during mastitis. Comparatively higher K_{12} and lower K_{21} in goats of all the experimental groups indicated that ceftriaxone is rapidly absorbed from the central compartment to the peripheral compartment and is slowly diffused back to the central compartment; which is bolstered by the lower F_c values of ceftriaxone in plasma, which shows the tendency of ceftriaxone to accumulate more in the tissue compartment. The higher *T~P* values of ceftriaxone in goats of all the groups also substantiate the above findings. From the above results it is observed that ceftriaxone in goats was increased by the presence of sulbactam and mastitis; hence, the combination therapy of ceftriaxone and sulbactam may be given preference to achieve the desired plasma level above the *MIC* than either of them alone. The Semi logarithmic plot of plasma concentration of metabolite ceftizoxime against time expressed 1-compartment model. Metabolite ceftizoxime persisted in the blood for longer period in mastitis induced goats following administration of ceftriaxone combined with sulbactam, which may be due to lower CL_B and higher $t_{1/2}\beta$ values. Ceftriaxone was rapidly metabolized to ceftizoxime after ceftriaxone dosing and was detected in the plasma for considerable period of time. The presence of the metabolite ceftizoxime in blood may enhance the antibiotic effect by preventing systemic infections which should be subjected for further therapeutic efficacy studies in future.

While drawing the therapeutic benefits of any antibiotic, β-lactamase inhibitor combination, it is very important that a β-lactamase inhibitor should also have a very similar pharmacokinetic behavior to the antibiotic component. Since, the semi logarithmic plot of plasma concentration of sulbactam against time had 2-compartment model, similar to the kinetics of parent drug ceftriaxone makes it highly suitable for the combination therapy. Study of Espuny *et al*., (1996) also reported that ampicillin: Sulbactam was distributed according to an open 2-compartment model after intra-venous administration. Even in experimental mastitis, significant amount of sulbactam was detected in plasma with the highest *AUC* value in combination therapy, suggesting mastitis does not alter sulbactam availability. However, experimental mastitis goats treated with sulbactam alone had serious depression and total in-appetence from the second day after mastitis challenge; hence, all these goats received fluid therapy (Ringers lactate) from the second day on wards. It was further noticed that these goats had grave clinical signs from third day onwards post challenge, and showed signs of recumbancy and decompensatory shock. Hence, were removed from the experiment after day three and were given intensive treatment with enrofloxacin, continuous intravenous fluids and other supportive therapy.

Milk $t_{1/2}\beta$ values (**Table1&2**) indicates ceftriaxone elimination slows up, from the mammary gland with sulbactam combination in experimental mastitis. The significantly lower AUC_{milk}: AUC_{plasma} of ceftriaxone in milk of mastitis challenged goats indicates lesser availability of ceftriaxone into udder quarters during mastitis, giving a possibility of antibiotic destruction by the bacterial load. The lower $C_{max\ milk}$: $C_{max\ plasma}$ (≤1) values indicated poor distribution and penetration of ceftriaxone into the mammary glands of mastitis goats as compared to normal lactating goats. Goudah *et al.,* (2006) also reported that concentrations of ceftriaxone in milk produced by clinically normal mammary glands of ewes were consistently lower than in serum. Longer mean residence time (*MRT*) of ceftriaxone in milk of goats in experimental mastitis indicates, mastitis increases the duration of ceftriaxone availability, especially in combination therapy, which is an encouraging factor for its therapeutic selection. Although there was significant increase in *P.P.B.* and *M.P.B*. in anti-mastitis ceftriaxone-sulbactam combination therapy which may support the above statement, but presence of inflammation may also alter the protein binding capacity. The bio availability of the ceftriaxone in the mammary gland of goats for longer period is an encouraging result to select the same drugs for the treatment of mastitis. Experimental mastitis enhanced metabolite ceftizoxime presence for a longer period in milk in combination therapy as it had higher $t_{1/2}\beta$ for the same group. Significantly lesser AUC_{milk}: AUC_{plasma} and $C_{max\ milk}$:$C_{max\ plasma}$ (≤1) ratio clearly indicates less availability and penetration of ceftizoxime in mammary glands during experimental mastitis. However, the presence of an active antibiotic metabolite may increase the overall efficacy of the parent drug ceftriaxone during mastitis by its additive effect, which should be exposed to further detailed efficacy studies. β-lactamase inhibitor, sulbactam in udder of goats was found to be lowest in experimental

mastitis which may be due to destruction of sulbactam in the mammary gland by the bacterial load producing β lacatamses. The higher milk Vd_{area} of sulbactam in combination with ceftriaxone suggests wider distribution in mammary gland of healthy as well as in experimental mastitis of goats. Hence, sulbactam and ceftriaxone combination therapy may be more effective in the treatment of mastitis. The comparatively lower AUC_{milk}: AUC_{plasma} and $C_{max\ milk}$:$C_{max\ plasma}$ (≤1) values indicates poor penetration of sulbactam into the mammary glands after induction of mastitis. The reason may be either increase in the pH of the milk in mastitis and sulbactam being a weakly acidic drug may not be able to cross the milk blood barrier or it may be due to destruction of the sulbactam due to β lactamases produced by the coliforms in the mammary gland.

Drug dosage regimen

The persistence of antibiotic concentrations in serum and tissues above *MIC* is the pharmacodynamic variable related to the clinical efficacy of ceftriaxone (Knudsen *et al.,* 1995; Soriano *et al*., 1996; Lustar *et al*., 1997). According to the excellent availability obtained in milk and a relatively low MIC determined in vitro, we consider ceftriaxone: sulbactam combination as a potential antimastitic drug and especially an alternative for the treatment of *Escherichia coli* mastitis in goats. In this study the values of C_{max} after intravenous administration exceeded the recommended susceptibility break point for ceftriaxone of 8 µg mL (NCCLS, 1999) by more than 10 times. Hence, the calculated dosage regimen (**Table 3**) may be effective in combating experimental mastitis caused by *Escherichia coli* in goats and the suggested treatment regimen may enhance the chance of successful treatment of life threatening mastitis in goats. However, considering the field conditions of veterinary practice, a ceftriaxone: sulbactam (1:1) dose of 8 mg Kg^{-1} bw in every 8 hours may be sufficient in the treatment of life threatening coliform mastitis in goats, where alternative therapies are not available.

Drug	MTPC (µg mL^{-1})	Animal Status	Group	Type of therapy	D_L (mg kg^{-1})	D_M (mg kg^{-1})	Γ (h)	$C_{ss\ avr}$ (µg mL^{-1})
Ceftriaxone	5-10	Healthy	Healthy Group I CTX	Single	4.1±0.55	2.75±0.23	2	7.2
Ceftriaxone	5-10	Mastitis	Mastitis Group I CTX	Single	6.26±0.92	4.25±0.36	3	7.2
Ceftriaxone	2-4	Healthy	Healthy Group III CTX:SUL	Combination	3.13±0.32	2.1±0.24	3	2.8
Ceftriaxone	2-4	Mastitis	Mastitis Group III CTX:SUL	Combination	1.28±0.21	0.92±0.11	2.5	2.8
Sulbactam	2-4	Healthy	Healthy Group III CTX:SUL	Combination	2.99±0.24	1.27±0.17	3	2.8
Sulbactam	2-4	Mastitis	Mastitis Group III CTX:SUL	Combination	1.36±.011	0.91±0.01	2.5	2.8

Table 3: Drug dosage regimen for ceftriaxone during single and combination therapy (Ceftriaxone: Sulbactam, 1:1) in healthy and experimental mastitis of black Bengal goats†.

MTPC- Minimum Therapeutic Plasma Concentration; D_L- Loading dose; D_M- Maintenance dose; Γ- Dosing interval; $C_{ss\ avr}$ – Steady state average plasma concentration

4. CONCLUSION

From the study, it may be concluded that a combination therapy of ceftriaxone with sulbactam in the ratio 1:1 persisted in the blank Bengal goats for longer time than either of them alone and the dosage of the drugs should be reduced in mastitis goats as compared to healthy goats. The study also endorses that ceftriaxone is rapidly metabolized into ceftizoxime. Ceftriaxone and sulbactam bind to the proteins of plasma and milk of healthy and mastitis induced goats when instituted either alone or in combination.

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