

Determination of ceftriaxone in plasma and ceftizoxime in milk of mastitic cows following single dose intravenous administration

T K SAR¹, T K MANDAL², I SAMANTA³, A RAHAMAN⁴ and A K CHAKRABORTY⁵

West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal 700 037 India

Received: 3 May 2010; Accepted: 21 September 2010

Key words: Ceftizoxime, Staphylococcus

National mastitis council (NMC) estimated an overall loss to dairy industry of 2 billion dollar (approximately 180 dollar per cow) due to mastitis (NMC 2005). Mastitis causes annual losses of over Rs 6 000 crore to India's dairy sector of which Rs 1 700 crore are due to clinical mastitis and Rs 4 400 crore due to sub clinical mastitis (Financial Daily 2002). One of the main problems in the selection of antibiotics for mastitis therapy on the basis of *in-vitro* sensitivity data is due to lack of pharmacokinetic data and drug concentration at the site of infection. Further sensitivity determination *in-vitro* may not reflect the effect in *in-vivo* condition.

The bacteria most commonly associated with bovine mastitis can be broadly divided into 2 groups: (a) those which need the animal for persistence—these bacteria multiply in the udder, teat canal or on skin; and (b) those 'opportunistic pathogens' which survive and multiply in extramammary sites (Quinn *et al.* 1994). It is a dynamic and fluctuating disease in which infection and inflammation wax and wane. Acute mastitis typically converts to a chronic subclinical carrier state of infection in which the host responses are suppressed (Sandholm *et al.* 1990).

Ceftriaxone, a third generation cephalosporin is used for the treatment of lower respiratory tract infections, urinary tract infections, gonorrhoea, peritonitis, skin and soft tissue infections and septicaemia caused by sensitive organisms. It is active against a wide range of gram-positive and gram-negative organisms. It is highly stable in presence of beta-lactamases, both penicillinases and cephalosporinases of gram-negative and gram-positive bacteria. The therapeutic milk concentration of gatifloxacin in mastitic goats (0.13 ± 0.05 to 12.41 ± 2.99 µg/ml) was maintained for 48 h (Singh

et al. 2010), while ceftizoxime, active metabolite of ceftriaxone persisted at a higher concentration for a longer period in milk of mastitic goats following single dose administration (Sar *et al.* 2006). Keeping this in view ceftriaxone was administered to cows, suffering from acute mastitis, to determine milk level of ceftriaxone and or ceftizoxime and pharmacokinetic behavior of ceftriaxone.

Case study: Two cows from Haringhata, Nadia and one from Magra, Hooghly of West Bengal were clinically examined. The cows suffering from acute mastitis exhibited inflammatory swelling and painful hot udder which secretes abnormal flakes in milk. This is also associated with fever, depression, anorexia, weakness and a rapid weak pulse.

Measurement of body weight of cow: To study the pharmacokinetics of ceftriaxone, body weight of mastitic cows was calculated as per Shaeffer's method (Sastry *et al.* 1982).

Collection of milk samples: Milk samples were collected from the cows in sterile vials aseptically and transported in ice immediately to the Microbiology Department, West Bengal University of Animal and Fishery Sciences. Samples were taken from the cows before the onset of any antimicrobial treatment.

Microbiological study of mastitic milk: Milk samples were centrifuged at 5 000 rpm for 10 min and Gram stained smear was prepared from the deposit. The deposits were further incubated overnight in the nutrient broth at 37°C. On the next day they were streaked into sheep blood agar and MacConkey agar and incubated at 37°C for overnight growth. Colonies were checked and single colony was transferred into nutrient agar slant for biochemical tests. The biochemical tests for detection of the species included catalase, oxidase, DNase, urease, maltose and mannitol fermentation. Pathogenic potentiality of the isolates was also judged by coagulase test (Quinn *et al.* 1994).

Intravenous administration of antimicrobial: A single dose of ceftriaxone dissolving in 10 ml of distilled water was administered through jugular vein at 2.55 mg/kg body weight.

Present address: ¹Assistant Professor (e mail: tapas.sar@rediffmail.com), ²Professor (e mail: drtkm48@gmail.com), ⁴PG Student (e mail: drakc75@yahoo.com), ⁵Ex-Reader (e mail: drakc75@yahoo.com), Department of Veterinary Pharmacology and Toxicology; ³Assistant Professor (e mail: isamanta76@gmail.com), Department of Veterinary Microbiology.

Johal and Srivastava (1999) and Dardi *et al.* (2004) administered ceftriaxone at 10 mg/kg body weight to cow calves and buffalo calves, respectively. Ceftizoxime persisted at a higher concentration for a longer period in milk of goats following single dose intravenous administration (Sar *et al.* 2006) which encouraged to administer ceftriaxone at a lower dose rate in mastitis to get adequate concentration in milk as ceftizoxime concentration may maintain therapeutic level for longer period in milk.

Collection of samples: Blood samples were collected at 0 and at 0.08, 0.16, 0.25, 0.5, 0.66 and 1 h period of internal (pd). Milk samples were collected at 0 and at 1, 2, 24, 48 and 72 h pd.

Estimation by high performance liquid chromatography: Assay of ceftriaxone and ceftizoxime was done as per Sar *et al.* (2006) and mobile phase was used as mentioned in United States Pharmacopia (USP).

Pharmacokinetic data analysis: The pharmacokinetic parameters were analyzed from computerized pharmacokinetic programme.

Microbiological study of mastitic milk: *Staphylococcus aureus* and *Staphylococcus albus* were detected from the milk of the mastitic cows. *Staphylococcus aureus* was coagulase positive. Coagulase positive *Staphylococcus aureus* was always reported in acute bovine mastitis (Petrovski *et al.* 2009). However coagulase negative *Staphylococcus* was also found to be associated with subclinical bovine mastitis (Thorberg *et al.* 2009). This support our present observation about the isolation of coagulase positive *Staphylococcus aureus* and coagulase negative *Staphylococcus albus* from crossbred mastitic cows. No attempt has been made to identify the isolates except staphylococci.

Plasma level of ceftriaxone: Mean values with SE of plasma concentration of ceftriaxone after single dose intravenous administration at 2.55 mg/kg in mastitic cows are presented in Table 1. Plasma concentration of ceftriaxone could be recorded at 0.08 h (23.76 ± 3.68 mg/ml), declined sharply and persisted up to 0.50 h pd (1.89 ± 0.44 mg/ml).

Kinetics: The semilogarithmic plot of mean plasma level time profile and mean kinetic parameters of ceftriaxone in mastitic cows after single dose intravenous administration at 2.55 mg/kg are presented in Table 2. Mean λ and $t_{1/2}$

Table 1. Mean plasma concentration (μ g/ml) of ceftriaxone in mastitic cows after single dose intravenous administration at 2.55 mg/kg (mean of 3 replicates with SE)

Time (hr)	Mean $\pm$ SE
0.08	23.76 ± 3.68
0.16	12.90 ± 2.57
0.33	5.13 ± 1.02
0.50	1.89 ± 0.44
0.66	BDL

BDL, Below detection limit.

Table 2. Mean kinetic parameters of ceftriaxone in mastitic cows after single dose intravenous administration of ceftriaxone at 2.55 mg/kg (mean of 3 replicates with SE)

Kinetic parameters	Mean $\pm$ SE
λ (h)	5.99 ± 0.39
$t_{1/2}$ (h)	0.11 ± 0.005
B (μ g/ml)	36.06 ± 5.83

Table 3. Mean milk concentration (mg/ml) of ceftizoxime in mastitic cows after single dose intravenous administration of ceftriaxone at 2.55 mg/kg (mean of 3 replicates with SE)

Time (h)	Mean $\pm$ SE
1	BDL
2	BDL
12	2.66 ± 1.20
24	10.00 ± 2.88
48	40.00 ± 7.63
72	69.41 ± 8.26
96	NC

BDL, Below detection limit; NC, not collected.

values were 5.99 ± 0.39 /h and 0.11 ± 0.005 h. Dardi *et al.* (2004) administered ceftriaxone at 10 mg/kg body weight to buffalo calves following a single dose intravenous administration and reported an elimination half-life was 3.79 ± 0.09 h. However in *E. coli* polysaccharide induced fever in buffalo calves, the elimination half-life was reported as 2.04 ± 0.14 h (Dardi *et al.* 2005). It is evident from the finding that elimination half life of ceftriaxone was decreased in *E. coli* polysaccharide induced fever. So, the lower $t_{1/2}$ value of ceftriaxone in the present experiment may be due to age variation, disease condition like mastitis and alteration in protein binding.

Milk level of ceftizoxime: Mean values with SE of milk concentration of ceftizoxime in mastitic cows after single dose intravenous administration of ceftriaxone at 2.55 mg/kg are incorporated in Table 3. Ceftizoxime was present at a concentration of 2.66 ± 1.20 mg/ml at 12 h pd in milk of mastitic cow, increased with advancement of time and showed a concentration of 69.41 ± 8.26 mg/ml at 72 h pd. Milk AUC₂₄ of ceftizoxime was 96.06 mg h/ml with peak concentration of 69.41 mg/ml and MIC as reported by Greenwood *et al.* (1980) for *Staphylococcus aureus*. The time above MIC ($T > MIC$) of ceftizoxime in milk was calculated to be 85% which suggests that the drug is highly effective against *Staphylococcus aureus* infection.

So it may be concluded that ceftizoxime, not the ceftriaxone is penetrating inflamed mammary gland of mastitic cows at a higher degree. Ceftizoxime being an amphoteric drug may cross blood milk barrier at a faster rate as the pH of the milk become alkaline during mastitis. Ismail

(2005) detected lower concentration of ceftriaxone in milk of goats following single dose intravenous and intramuscular administration at 20 mg/kg body weight. Goudah *et al.* (2006) also reported lower concentration of ceftriaxone in milk of lactating ewes following single dose intravenous and intramuscular administration at 10 mg/kg body weight and the kinetic value AUC_{milk}/AUC_{serum} and $C_{max\ milk}/C_{max\ serum}$ ratios was <0.4 . These low values indicated poor distribution and penetration of ceftriaxone from the bloodstream to the mammary gland of lactating ewes following both routes. In the present experiment, ceftriaxone was present at below detectable level as the dose rate was only 2.55 mg/kg body weight and the production of milk was comparatively higher in cows. Therefore, the difference of observed data between previous work and the present one may be attributed towards the poor penetration of ceftriaxone from blood to mammary gland.

SUMMARY

Cows (3) suffering from acute mastitis were given a single dose of ceftriaxone at 2.55 mg/kg bwt. Culture of milk showed the presence of *Staphylococcus aureus* and *Staphylococcus albus*. Distribution of ceftriaxone and its active metabolite were calculated from both plasma and milk. Plasma concentration of ceftriaxone was first recorded at 0.08 h (23.76 ± 3.68 microgram/millilitre) which then declined sharply. Mean elimination rate constant and biological half life values were $5.99 \pm 0.39/h$ and 0.11 ± 0.005 h, respectively. Ceftizoxime as a major active metabolite of ceftriaxone was identified for first time in the milk of mastitic cows.

ACKNOWLEDGEMENT

The authors are grateful to Professor Anjan Bhattacharyya, In-Charge APEDA and Head, Department of Agricultural Chemicals, Bidhan Chandra Krishi Viswavidyalaya (BCKV), for providing HPLC to carry out the estimation of studied drugs.

REFERENCES

- Dardi M S, Sharma S K and Srivastava A K. 2004. Pharmacokinetics and dosage regimen of ceftriaxone in buffalo calves. *Veterinary Research Communications* **28**: 331–38.
- Dardi M S, Sharma S K and Srivastava A K. 2005. Pharmacokinetics and dosage regimen of ceftriaxone in *E. coli* lipopolysaccharide induced fever in buffalo calves. *Journal of Veterinary Sciences* **6**:147–50
- Financial Daily. 2002. *Mastitis: Expert Calls for Early Detection*. Hindu Group of Publications, Tuesday, September 10.
- Goudah A, Shin H C, Shim J H and Abd Elaty A M. 2006. Characterization of the relationship between serum and milk residue disposition of ceftriaxone in lactating ewes. *Journal of Veterinary Pharmacology and Therapeutics* **29**: 307–12.
- Greenwood D, Pearson N, Eley A and O'Grady F. 1980. Comparative *in vitro* activities of cefotaxime and ceftizoxime (FK749): New cephalosporins with exceptional potency. *Antimicrobial Agents and Chemotherapy* **17**: 397–401.
- Ismail M M. 2005. Pharmacokinetics, urinary and mammary excretion of ceftriaxone in lactating goat. *Journal of Veterinary Medicine Series A* **52**: 354–58.
- National Mastitis Council 2005. NE-1009, *USDA Multistage Research Project, Questions and Comments*: w-hurley@uiuc.edu.
- Petrovski K R, Heuer C, Parkinson T J and Williamson N B. 2009. The incidence and aetiology of clinical bovine mastitis on 14 farms in Northland, New Zealand. *New Zealand Veterinary Journal* **57**:109–15.
- Quinn P J, Carter M E, Markey B K and Carter G R. 1994. *Clinical Veterinary Microbiology*. 1st edn. Wolfe Publications, UK.
- Sandholm M, Kaartinen L and Pyaerc S. 1990. Bovine mastitis-why does antibiotic therapy not always work? An overview. *Journal of Veterinary Pharmacology and Therapeutics* **13**: 248–60.
- Sar T K, Mandal T K, Das S K, Chakraborty A K and Bhattacharyya A. 2006. Pharmacokinetics of ceftriaxone in healthy and mastitic goats with special reference to its interaction with polyherbal drug Fibrosin®. *International Journal of Applied Research in Veterinary Medicine* **4**:142–54.
- Sastry N S R, Thomas C K and Singh R A. 1982. *Farm Animal Management and Poultry Production*. 2nd edn. Vikas Publishing House Pvt. Ltd., New Delhi
- Singh V, Ram M, Roy B K and Singh K K. 2010. Effect of induced mastitis on disposition kinetics of gatifloxacin following Intravenous administration in goats. *Journal of Bioanalysis and Biomedicine* **2**: 44–48.
- Thorberg B M, Danielsson-Tham M L, Emanuelson U and Persson Waller K. 2009. Bovine subclinical mastitis caused by different types of coagulase-negative staphylococci. *Journal of Dairy Science* **92**:4962–70.