Indian Journal of Animal Sciences **86** (2): 140–142, February 2016/Article
https://doi.org/10.56093/ijans.v86i2.55774

Disposition kinetics of ceftizoxime in acute mastitis in Murrah buffaloes

RAKESH KUMAR[1], T K SAR[2], P H PATRA[3], I SAMANTA[4], AKHILESH MISHRA[5] and T K MANDAL[6]

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

Received: 5 November 2014; Accepted: 7 July 2015

ABSTRACT

Acute mastitis was induced by inoculation of *Staphylococcus aureus* in 6 buffaloes. Ceftizoxime was administrated intravenously @25 mg/kg to evaluate pharmacokinetics of ceftizoxime in mastitic buffaloes. The maximum mean plasma concentration of ceftizoxime was recorded at 0.08 h in healthy buffaloes which was followed by decreased plasma concentration of 23.53 ± 0.84 µg/ml at 1 h and persisted up to 24 h. Mean values of t½ß were 5.86 ± 0.18 and 8.42 ± 0.58 h in healthy and induced mastitic buffaloes, respectively. Mean Vd_{area} value of 1.10 ± 0.06 L/kg in healthy buffaloes was increased significantly. The maximum mean milk concentration of ceftizoxime at 2 h (73.34 ± 4.35 µg/ml) in lactating buffaloes decreased gradually till 12 h (31.31 ± 5.66 µg/ml) and persisted up to 96 h (1.57 ± 0.20 µg/ml). So, ceftizoxime can be used for treatment of staphylococcal mastitis in buffaloes.

Key words: Buffalo, Ceftizoxime, Mastitis, Milk level, Pharmacokinetics

Buffalo mastitis caused by *Staphylococcus aureus* incurs substantial economic losses to the dairy industry worldwide. Veterinarians are useing ceftizoxime, a third generation cephalosporin, which is highly stable to ß lactamases. Pharmacokinetics of ceftriaxone was reported in goats and cows in mastitic condition, and active metabolite of ceftriaxone i.e. ceftizoxime persisted for a longer period in milk (Sar *et al.* 2006, 2010). Therefore the present study was aimed to explore the pharmacokinetics of ceftizoxime in acute staphylococcal mastitis in buffaloes.

MATERIALS AND METHODS

Ceftizoxime (technical grade, purity ³ 90%), the test drug was obtained commercially. Clinically 6 healthy lactating Murrah buffaloes of 4 to 5 years of age, approximately weighing 305 to 345 kg were used for the experiment. All animals were stall fed with chopped paddy straw and concentrate mixture. Green fodders like berseem, hybrid napier grass were supplied with *ad lib.* drinking water. The animals were dewormed with single oral dose of levamisole and triclabendazole at 1 bolus 100/kg 28 days prior to onset of study. Before the start of the experiment, the animals were acclimatized for 7 days. During this period, body temperature, pulse and respiration rate, quantity of milk were recorded daily. All buffaloes showed milk enzyme activity in the normal range and negligible number of bacteria.

Healthy buffaloes (6: control/group 1) and induced mastitic buffaloes (group 2) were given a single dose of ceftizoxime @25 mg/kg body weight intravenously to evaluate pharmacokinetics of ceftizoxime in healthy buffaloes. The same buffaloes were utilized for induction of experimental staphylococcal mastitis allowing 1 month wash-out period. The whole experiment protocol was approved by Institutional Animal Ethical Committee. *Staphylococcus aureus* was isolated and identified from mastitic milk samples of buffaloes using standard technique (National Mastitis Council 1999). The experimental 6 buffaloes were inoculated intracisternally to rear left quarter with the isolated *S. aureus* (1×10^8 c.f.u./ml).

Collection of samples: Blood samples (2 ml) were collected from the jugular vein in heparinized test tubes at '0' (before drug administration) and at 0.08, 0.16, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, and 120 h post drug administration. Plasma was separated and 1 ml was utilized for the analysis of ceftizoxime concentration. Milk samples (2 ml) were collected from both rear and front teats into the test tube at '0' (before drug administration) and at 0.08, 0.16 ,0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, and 120 h post drug administration. Milk samples were kept in deep freeze until analysis. Milk samples taken from both rear and front teats, mixed and 1 ml were taken for estimation of drug concentration. The remaining milk sample was utilized for estimation of some enzymatic activity.

Extraction of ceftizoxime from plasma/ milk: Extraction of ceftizoxime from plasma/ milk was done according to

Present address: [1]PG Scholar (drrakeshkumar30@gmail.com), [2](tapas.sar@rediffmail.com), [5]Professor and Head (drtkm48 @yahoo.co.in), Department of Pharmacology and Toxicology, [4]Assistant Professor (isamanta76@gmail.com), Department of Veterinary Microbiology. [3]Assistant Professor (drphpatra @gmail.com), Department of Pharmacology and Toxicology, College of Veterinary Sciences and Animal Husbandry, Agartala, Tripura.

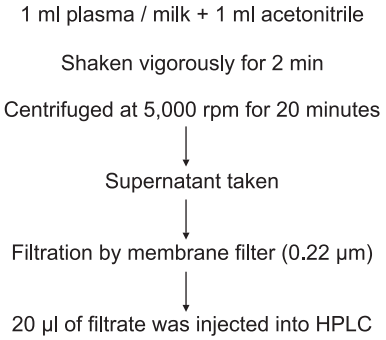

Fig. 1. Flow chart for extraction of ceftizoxime from plasma and milk.

Sar *et al.* (2011) (Fig. 1) and estimation of ceftizoxime from both plasma and milk was done using liquid chromatograph coupled with photo diode-array detector (UV-VIS) attached with computer SPD MXA 10 software. A 5µ Luna C18 (2); 250 × 4.6 mm (RP) column was used with the flow rate of 1.5 ml/min and 254 nm wavelength. The mobile phase was prepared according to United States Pharmacopoeia. Standard and sample (20µl) were injected into the injector port of liquid chromatograph with the first and last being the standard. The drug was estimated after comparing with external standard. Calibration was done every time by a standard stock solution of 20 ppm of the test drugs of analytical grade prepared in distilled water. Recovery of ceftizoxime from plasma/milk was carried out *in vitro.* The recovery percentage of ceftizoxime were 81.14 ± 4.95% and 79.83 ± 4.21% in plasma and milk, respectively. The limit of detection for both the drugs in plasma and milk was 0.01 ppm. Pharmacokinetic parameters of ceftizoxime were determined as per Baggot (1977). The data were analyzed by paired t-test and t-test of independent sample assuming equal variance using SPSS 10.0. The results were expressed as mean ± standard error (S.E.).

RESULTS AND DISCUSSION

Animals showed the symptoms of anorexia, slightly increased temperature (103-104°F), swollen, hard and hot udder which was painful on touch. Initially, there was cessation of milk followed by brown yellow fluid with flakes. The whole udder showed the signs of mastitis following intracisternal inoculation of *S. aureus* into the rear left quarter, which is in consistent with earlier report (Sar *et al.* 2006). A confirmatory test was performed using BTB (Bromothymol blue) paper test (Table 1).

The maximum mean concentration of ceftizoxime

Table 1. Colour reaction in BTB paper test

Time (h)	Colour of milk	
	Hind quarter	Front quarter
24	Light green	Light green
48	Dark green	Bit dark green
72	Deep bluish green	Dark green

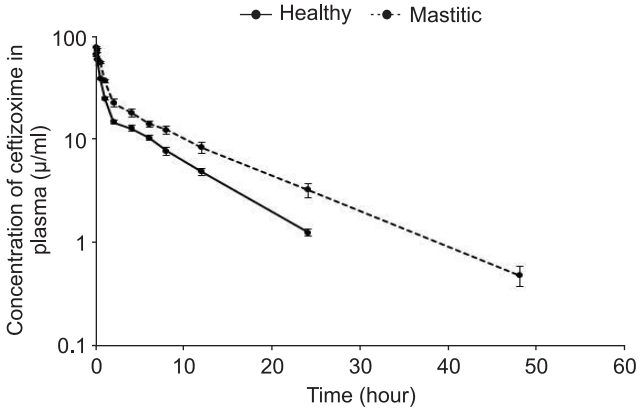

Fig. 2. Semi logarithmic plot of mean plasma concentration of ceftizoxime in healthy lactating and induced mastitic buffaloes after single dose of intravenous administration at 25 mg kg^{-1} body weight.

(61.87±1.75 µg/ml) was recorded at 0.08 h in lactating buffaloes, which decreased gradually till 1 h with a plasma concentration of 23.53±0.84 µg/mland persisted up to 24 h with a concentration of 1.23±0.16 µg/ml. The maximum mean concentration of ceftizoxime in plasma was 73.54±1.31µg/ml at 0.08 h in induced mastitic buffaloes, which decreased to 35.02±1.92 µg/ml at 2 h and persisted up to 48 h. Ceftizoxime persisted for longer period in plasma and milk of mastitic buffaloes, which may be due to the particular disease condition. The kinetic profile of ceftizoxime in healthy lactating and mastitic buffaloes followed "two compartment open model" (Fig. 2). The mean values of t½á and t½ ß were 0.32±0.01 h and 5.86±0.18 h, respectively, in healthy lactating buffaloes while the mean of t½ á and t½ ß were 0.53±0.02 h and 8.42±0.58 h, respectively, in mastitic buffaloes indicating the longer persistence of ceftizoxime in mastitis. Mean K_{12} value (1.19±0.06/h) was significantly higher ($P<0.05$) compared to K_{21} (0.67±0.02/h) indicating higher distribution of ceftizoxime from central to peripheral compartment in healthy group. The Vd_{area} value of 1.10±0.06 L/kg indicated wide distribution of ceftizoxime in healthy lactating buffaloes. The mean Cl_B value of 0.97±0.001 L/kg/h in healthy buffaloes suggested comparatively slower elimination of ceftizoxime from body. The mean AUC, Vd_{area}, Cl_B and MRT values in mastitic buffaloes were 330.56±29.23 µg/ml, 0.45±0.10 L/kg, 1.49±0.29 L/kg/h and 10.50±0.69 h, respectively. The mean Vd_{area} value of 0.95±0.10 L/kg indicated wide distribution of ceftizoxime in induced mastitic buffaloes.

Ceftizoxime persisted for 48 h at a concentration of 0.48±0.14 µg/ml in plasma of mastitic buffaloes compared to 24 h in healthy buffaloes suggesting longer persistence of the drug in plasma of mastitic buffaloes. In corroboration, Sar *et al.* (2006) evaluated pharmacokinetic profile of ceftriaxone in healthy and mastitic goats following single dose intravenous administration and identified ceftizoxime as a major active metabolite of ceftriaxone, which persisted from 36 to 96 h pd in plasma of polyherberal-treated goats following single dose intravenous administration. Plasma

concentration of ceftizoxime was also significantly higher in mastitic buffaloes compared to healthy buffaloes at different time intervals. Mean t½ß value of 8.42±0.58 h significantly increased in mastitic buffaloes suggesting longer persistence of ceftizoxime in plasma which may also be responsible for comparatively longer persistence of the drug in milk. Mean AUC value was also significantly increased in mastitic buffaloes suggesting wide coverage of distribution even in the peripheral organ like mammary gland.

The maximum mean milk concentration of ceftizoxime was achieved at 2h (73.34±4.35 µg/ml) in healthy lactating buffaloes, which decreased gradually till 12 h (31.31±5.66 µg/ml) with declining of milk concentration bit by bit and persisted up to 96 h with a concentration of 1.57± 0.20 µg/ml. On the other hand, milk concentration of 24.88±1.30 µg/ml at 0.08 h was followed by an increase in milk concentration with advancement of time and showed a maximum concentration of 78.09±6.58 µg/ml at 2 h post administration in induced mastitic buffaloes. The milk concentration of ceftizoxime started to decline gradually from 4 h and persisted up to 120 h with a concentration of 0.81±0.02 µg/ml. The disposition of ceftizoxime followed 'two compartment open model' in healthy and induced mastitic buffaloes. Mean t½Ka values were 0.66±0.16 h and 0.84±0.20 h in healthy and mastitic buffaloes, respectively. Mean t½Ka value did not alter significantly between 2 groups, but t½ß value of 14.99±0.36 h in healthy buffaloes decreased significantly compared to t½ß value (21.57 ± 1.50 h) in mastitic buffaloes (Table 2).

Mean milk concentration of ceftizoxime was significantly higher starting from 4 h to 72 h post dosing in mastitic buffaloes, while the drug persisted for 120 h pd in mastitic buffaloes compared to 96 h pd in healthy buffaloes. The higher concentration of ceftizoxime in milk of mastitic buffaloes could be described by the pH partition hypothesis (Baggot 1977). During mastitic condition, pH of the milk increases and becomes alkaline in nature. Ceftizoxime being an amphoteric drug may attain higher concentration in alkaline milk due to ion trapping. Karmakar *et al.* (2011) also reported a higher milk concentration of ceftizoxime in mastitic goat than healthy ones following single intravenous dosing. Longer persistence of ceftizoxime in both the groups corroborates with the earlier reports of Sar *et al.* (2006) and Karmakar *et al.* (2011). Prolonged persistence of ceftizoxime in milk in mastitic cow was reported following single intravenous dosing of ceftriaxone (Sar *et al.* 2010).

So, ceftizoxime can be administered by intravenous route for effective treatment of *S. aureus* mastitis in buffaloes as the drug persists for a significantly longer period (t½ß: 21.57 ± 1.50 h) compared to healthy buffalo (t½ß: 14.99 ±0.36 h). As it is evidenced from the present study that milk concentration of ceftizoxime was also significantly higher starting from 4 h to 72 h post dosing in mastitic buffaloes, while the drug persisted for 120 h pd in mastitic buffaloes compared to 96 h pd in healthy buffaloes following single intravenous dosing of ceftizoxime @25 mg/kg body weight.

Table 2. Mean kinetic parameters of ceftizoxime in milk of healthy lactating and induced mastitic buffaloes after single dose intravenous administration at 25 mg/kg body weight (mean with SE of 6 replicates)

Kinetic parameter	Healthy buffaloes	Mastitic buffaloes
Ka/h	0.66 ± 0.16	0.84 ± 0.24
MRT (h)	23.70±0.74	27.69*±0.34
Vd area (L/kg)	0.51±0.06	0.37±0.04
AUC (µg h/ml)	1633.40±138.05	2392.12*±73.21
ß/h	1.38±0.30	0.82*±0.13
t½Ka (h)	0.10 ± 0.069	0.03 ± 0.03
t½ß (h)	14.99 ±0.36	21.57** ± 1.50
ClB (L/kg/h)	0.02±0.004	1.16**±0.001

*, Significant differences between rows (P<0.05);**, significant differences between rows (P<0.01). Ka, absorption rate constant; MRT, minimum retention time, Vdarea : Apparent volume of distribution (area method); AUC, total area under the plasma ceftizoxime concentration versus time curve; ß, elimination half life; t1/2 Ka, absorption half life; t1/2ß, elimination half life; ClB, total body clearance of ceftizoxime.

REFERENCES

Baggot J D. 1977. Principles of Drug Disposition in Domestic Animals. *The Basis of Veterinary Clinical Pharmacology*, W. B. Saunders Co., Philadelphia, London.

Karmakar U, Datta B K, Patra P H, Prashant M, Suman S, Sar T K, Chakraborty A K and Mandal T K. 2011. Dispostion kinetics of ceftizoxime in healthy and mastitic goats after intravenous administration. *Pharmacology online* **3**: 935–46.

National Mastitis Council. 1999. *Laboratory Handbook on Bovine Mastitis*. Inc., Madison, Wisconsin, USA. 44p.

Sar T K, Mandal T K, Samanta I, Rahaman A and Chanraborty A K. 2010. Determination of ceftriaxone in plasma and ceftizoxime in milk of mastitic cows following single dose intravenous administration. *Indian Journal of Animal Sciences* **80**: 1182–84.

Sar T K, Mandal T K, Das S K, Chakraborty A K and Bhattacharyya A. 2006. Pharmacokinetics 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.

Sar T K, Patra P H, Dash J R and Mandal T K. 2011. Pharmacokinetic interaction of intramammary ceftriaxone and oral polyherbal drug (Fibrosin ®) in goats. *Drug Metabol Drug Interact* **26**: 191–96.