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Research Article

MILK LEVEL OF CEFTIZOXIME FOLLOWING SINGLE INTRAMUSCULAR DOSING OF CEFTRIAXONE WITHOUT AND WITH ORAL ADMINISTRATION OF FIBROSIN® AND ITS EFFECT ON MILK ENZYME ACTIVITY IN GOAT

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ABSTRACT: The veterinarians frequently use ceftriaxone intramuscularly in treatment of mastitis. Fibrosin® , a polyherbal drug is also commonly used as supportive therapy for let down of milk during mastitis. The present study was conducted to determine milk level of ceftizoxime, a major active metabolite of ceftriaxone and its effect on milk enzyme activity in lactating goats following single dose intramuscular administration of ceftriaxone (@ 50 mg / kg body weight) with or without one hour prior to oral administration of polyherbal drug (1.9 gm). Twelve clinically healthy lactating Black Bengal goats were divided into two groups (namely Group I, group II) each containing six goats. A single intramuscular dose of ceftriaxone was administered at 50 mg/kg body weight to each goat of group-I only, while a total dose of 1.9 gm of polyherbal drug was administered orally to each goat of group-II before one hour of cefriaxone administration. Milk concentration of ceftriaxone and ceftizoxime were analyzed by HPLC. Ceftizoxime was detected in milk from 5 minutes to 24 hours post dosing following single intramuscular dose of ceftriaxone without Fibrosin® administration. However, neither ceftriaxone nor ceftizoxime could be detected in milk following single intramuscular dosing of ceftriaxone with Fibrosin® administration. Milk alkaline phosphatase and catalase activity as well as reduced glutathione level did not differ significantly between group I and group II animals. Milk alkaline phosphatase activity was increased markedly following intramuscular administration of ceftriaxone indicating mammary tissue damage. Thus the present study showed that Fibrosin® should be avoided with intramuscular ceftriaxone for treatment of mastitis due to unavailability of ceftriaxone / ceftizoxime in milk. However, oral administration of Fibrosin can be preferred in other bacterial infections with concurrent administration of intramuscular ceftriaxone as it reduces milk residue of ceftizoxime.

Key words: Intramuscular Ceftriaxone, Mastitis, Polyherbal drug, Milk enzyme activity.

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INTRODUCTION

West Bengal has its own breed – Bengal goat, a unique versatile animal which produces excellent quality meat and easily digestible milk (Zeshmarani *et al.*, 2007). Keeping this in view, goat is considered as the experimental animal which is well accepted as a model ruminant for research purpose. The veterinarians frequently use ceftriaxone intramuscularly in treatment of mastitis. Fibrosin® , a polyherbal drug is also commonly used as supportive therapy for let down of milk during mastitis.

The present study was aimed to determine the milk level of ceftizoxime (active metabolite of ceftriaxone) following single intramuscular dosing of ceftriaxone in presence or absence of polyherbal drug. Further, the effect of combination therapy (oral Fibrosin® and intramuscular ceftriaxone) and only ceftriaxone therapy on milk enzyme activity was also evaluated.

MATERIALS AND METHODS

Experimental Animals

Twelve apparently healthy lactating Black Bengal goats were divided into two groups (namely Group I, group II) each containing six goats. The animals were caged individually in custom's made stainless steel metabolic cage and provided with *ad libitum* drinking water and standard feed.

Drug administration

A single dose of ceftriaxone dissolving in 5 ml of distilled water was administered at 50 mg kg^{-1} body weight intramuscularly in group I goats. A total dose of 1.9 gm of Fibrosin® [Kanchanar-gugal (Bauhinia veriegata Lin.), Chitrak-mula (Plumbago zeylanica), Punarnavastaka (Triaanthema monogyna), Trifala (*Terminalia belerica* Retz. + *Terminalia chebula Retz.* +*Phylanthus amblica*), Apamarga (*Achyranthes aspera* lin)] was administered orally 1 hr prior to intramuscular ceftriaxone administration in group II goats.

Collection of samples

Milk samples (2 ml) were collected from both the teat at '0' and at 0.08, 0.16, 0.25, 0.33, 0.50, 0.66, 1, 2, 3, 4, 6, 8, 12, 24, and 36 hr post dosing and 1 ml was utilized for determination of milk concentration of ceftriaxone / ceftizoxime. Further, milk samples were collected at 48, 72, 96 hr post dosing and stored at 4°C for determination of milk enzyme activity.

Estimation of milk enzyme activity

Estimation of reduced glutathione level, alkaline phosphatase, catalase activity in milk was done according to the method described by Pecker (1994), Bernt (1974), Maehly and Chance (1954), respectively.

Analysis of drug concentration

Estimation of Ceftriaxone / Ceftizoxime in milk was done by the method of Sar *et al.* (2011).

Statistical analysis

The mean and standard error as well as level of significance was analyzed by SPSS version 21 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Milk level of Ceftizoxime

Milk level of Ceftizoxime showed a zigzag pattern following single intramuscular dosing of Ceftriaxone. The Ceftizoxime concentration was 2.00 ± 0.28 µgm/ml at 0.08 hr post dosing which was markedly increased to 92.31 ± 3.69

Table 1.Mean milk concentration (µg ml^{-1}) of ceftizoxime in healthy lactating goats without and with 1 hr pre single dose oral administration of fibrosin (1.9 gm) after single dose intramuscular administration of ceftriaxone at 50 mg kg^{-1} (Mean of 3 replicates with SE).

Time (hr)	Healthy lactating (Gr I)	Fibrosin treated healthy lactating (Gr II)
0.08	2.00 ± 0.28	Below detectable level (BDL)
0.16	1.33 ± 0.33	BDL
0.25	20.33 ± 1.21	BDL
0.33	4.16 ± 1.01	BDL
0.50	92.31 ± 3.69	BDL
0.66	38.41 ± 2.60	BDL
1	12.01 ± 1.93	BDL
2	10.08 ± 1.30	BDL
3	3.70 ± 0.90	BDL
4	56.93 ± 4.67	BDL
6	177.86 ± 10.14	BDL
8	156.43 ± 10.78	BDL
12	126.00 ± 9.23	BDL
24	3.58 ± 1.01	BDL
36	BDL	BDL

µgm/ml at 0.50 hr and which was again decreased followed by increase in concentration and showed a maximum value of 177.86 ± 10.14 µgm/ml at 6 hr post dosing and persisted up to 24 hr post dosing at 3.58 1.01 µgm/ml in milk (Table 1). This may be due to absorption and reabsorption of ceftizoxime in the mammary gland following intramuscular dosing of ceftriaxone. Sar *et al.* (2011) also reported a zigzag pattern of ceftizoxime concentration in milk following intramammary administration of ceftriaxone with 1 hour prior oral administration of Fibrosin®. However, neither ceftriaxone nor ceftizoxime could be detected in milk following single intramuscular dosing of ceftriaxone with oral polyherbal drug Fibrosin®. The polyherbal drug Fibrosin® inhibited metabolism of ceftriaxone by reducing microsomal Cytochrome P_{450} level in liver of healthy goats following single intravenous dosing of ceftriaxone at 50 mg / kg body weight although ceftizoxime was detected in milk (Sar *et al.*, 2006). Bioavailability of any drug decreases following intramuscular dosing in comparison to intravenous dosing. Therefore, prior oral administration of Fibrosin® significantly inhibited metabolism of Ceftriaxone which may be responsible for unavailability of its active

Table 2. Mean alkaline phosphatase activity (n mole PNP produced hr^{-1} ml^{-1} of milk) in healthy lactating goats without and with 1 hr pre single dose oral administration of fibrosin (1.9 gm) after single dose intramuscular administration of ceftriaxone at 50 mg kg^{-1} (Mean of 3 replicates with SE).

Time (hr)	Healthy lactating (Gr I)	Fibrosin treated healthy lactating (Gr II)
0	$7524.00^{NS} \pm 1070.43$	$7840.00^{NS} \pm 499.42$
1	-	$9640.00^{NS} \pm 918.01$
24	$5148.00^{NS} \pm 285.79$	$6300.00^{NS} \pm 681.29$
48	$5544.00^{NS} \pm 628.75$	$9280.00^{NS} \pm 1001.73$
72	$23248.80^{NS} \pm 2500.46$	$10320.00^{NS} \pm 1174.94$
96	$40953.60^{NS} \pm 4372.17$	$12450.00^{NS} \pm 1677.25$

NS – Non-significant.

Table 3. Mean milk catalase activity (m mole H_2O_2 hydrolysed min^{-1} ml^{-1} of milk) in healthy lactating goats without and with 1 hr pre single dose oral administration of fibrosin (1.9 gm) after single dose intramuscular administration of ceftriaxone at 50 mg kg^{-1} (Mean of 3 replicates with SE).

Time (hr)	Healthy lactating (Gr I)	Fibrosin treated healthy lactating (Gr II)
0	$20.81^{NS} \pm 3.29$	$17.79^{NS} \pm 2.85$
1	-	$17.46^{NS} \pm 3.09$
12	$15.89^{NS} \pm 2.96$	$12.98^{NS} \pm 2.96$
24	$15.67^{NS} \pm 3.12$	$12.31^{NS} \pm 2.95$
48	$15.00^{NS} \pm 3.03$	$14.55^{NS} \pm 3.14$
72	$20.50^{NS} \pm 3.03$	$15.00^{NS} \pm 3.14$
96	$28.76^{NS} \pm 6.06$	$16.23^{NS} \pm 2.87$

NS – Non-significant.

metabolite *i.e.* ceftizoxime in milk following intramuscular dosing of ceftriaxone. Ceftriaxone showed absorption-reabsorption pattern in plasma following single intramuscular dosing (Sar *et al*., 2013). In the present study, ceftizoxime showed a zigzag pattern in milk following single intramuscular dosing of Ceftriaxone because its parent drug

Table 4. Mean reduced glutathione level (n mole GSH ml^{-1} of milk) in healthy lactating goats without and with 1 hr pre single dose oral administration of fibrosin (1.9 gm) after single dose intramuscular administration of ceftriaxone at 50 mg kg^{-1} (Mean of 3 replicates with SE).

Time (hr)	Healthy lactating (Gr I)	Fibrosin treated healthy lactating (Gr II)
0	$490.00^{NS} \pm 80.83$	$440.75^{NS} \pm 69.42$
1	-	$455.75^{NS} \pm 72.45$
8	$452.50^{NS} \pm 82.27$	$335.00^{NS} \pm 51.96$
24	$357.50^{NS} \pm 44.74$	$300.50^{NS} \pm 20.49$
48	$245.00^{NS} \pm 34.64$	$338.75^{NS} \pm 34.61$
72	$370.25^{NS} \pm 46.04$	$458.75^{NS} \pm 62.78$
96	$380.75^{NS} \pm 46.62$	$445.20^{NS} \pm 72.22$

NS – Non-significant.

i.e. ceftriaxone also showed absorption-reabsorption pattern in plasma (Sar *et al.*, 2013).

Estimation of milk enzyme activity

The normal milk alkaline phosphatase activity in healthy goats ranged from 7524.00 ± 1070.43 to 7840.00 ± 499.42 n mole para nitro phenol produced hr^{-1} ml^{-1} which was increased many fold $40953.60^{NS} \pm 4372.17$ n mole para nitro phenol produced hr^{-1} ml^{-1} at 96 hr following intramuscular dosing of ceftriaxone (Table 2). This finding strongly supports that intramuscular injection of ceftriaxone may cause substantial tissue damage which produces marked increase in milk alkaline phosphatase activity. The oral administration of polyherbal drug Fibrosin® did not alter the increased milk alkaline phosphatase activity significantly ($p<0.05$) in group II goats.

The normal milk catalase activity ranged from 20.81 ± 3.29 to 17.79 ± 2.85 (m mole H_2O_2 hydrolysed min^{-1} ml^{-1} which did not differ significantly at different time following intramuscular dosing of ceftriaxone in group I goats (Table 3). Administration of oral polyherbal drug did not affect milk catalase activity significantly in Group II goats also. The finding suggests that either ceftriaxone (antibacterial drug) or Fibrosin (polyherbal drug) has no influence on milk catalase activity.

The control value of reduced glutathione in milk ranged from 490.00 ± 80.83 to 440.75 ± 69.42 n mole reduced glutathione ml^{-1} which did not vary significantly following intramuscular dosing of ceftriaxone in group I goats as well as in group II goats with 1 hour prior oral administration of Fibrosin® (Table 4). Glutathione is a significant component of the collective antioxidant defenses and is highly potent antioxidant. Reduced glutathione is also essential both to the functional and structural integrity of the cells, the tissues and the organ system. The glutathione status of a cell (that is the excess of reduced over oxidized glutathione) will perhaps

turn out to be the most accurate single indicator of the health of a cell. In the present study, it was observed that intramuscular dosing of ceftriaxone markedly increase milk alkaline phosphatase activity whereas reduced glutathione level remained unaltered. So it can be concluded that though intramuscular injection of ceftriaxone caused udder tissue damage but it had no effect on functional integrity of the mammary tissue as reduced gluatathione level did not alter significantly. The polyherbal drug, Fibrosin should be better avoided with intramuscular dosing of ceftriaxone particularly for treatment of mastitis as neither ceftriaxone nor ceftizoxime was available in milk which produces anti-bacterial effect to combat the infection. However, oral administration of Fibrosin can be recommended with concurrent administration of intramuscular ceftriaxone for treatment of other bacterial infections as it reduces milk residue of ceftizoxime.

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