DISPOSITION KINETICS OF CEFTIZOXIME IN HEALTHY AND MASTITIC GOATS AFTER INTRAVENOUS ADMINISTRATION

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Runninig title: Ceftizoxime in healthy and mastitic goats

Summary

The study was conducted to fix the dosage regimen after intravenous administration of ceftizoxime, a third generation cephalosporin group antibiotic along with its efficacy in mastitis condition in goats. Twelve black Bengal goats were divided into two groups containing six animals each out of which mastitis was induced in the second group using pathogenic strain of *Escherichia coli*. Mastitis was confirmed by lactoperoxidase, catalase and bromothymol blue paper test. A single intravenous dose of ceftizoxime at 10 mg kg^{-1} was administered in both the groups. Blood and milk samples were collected at different time interval. Ceftizoxime was analyzed by HPLC. Mean $t_{½}$ β, AUC, Cl_B , and MRT were increased but Vd_{area} and K_{el} were decreased significantly ($P<0.01$) in mastitic goats compared to healthy goats. Ceftizoxime was persisted upto 144 hr post administration in mastitic goats in comparison to 96 hr post administration in healthy goats. Clinical manifestation of mastitis was cured and milk enzymes come to its normal level at 120 hr post administration of intravenous ceftizoxime administration. Mastitis alters significantly the disposition kinetics of ceftizoxime in healthy goats after intravenous administration. Dose and dosing interval of ceftizoxime should be reduced and increased respectively in mastitic goats compared to healthy goats.

Key words: Ceftizoxime, pharmacokinetics, intravenous, mastitis

Introduction

Clinical mastitis is recognized to cause major economic losses in dairy cattle. It is estimated that 70% of the economic losses from this disease is caused by lower milk production, 14% to premature animal removal, 7% to milk wasting and 8% to veterinary expenses and treatments [(1)]. An annual loss of Rs. 4396.32 crores due to staphylococcal mastitis in India has been reported [(2)]. Cephalosporin antibiotics are being used more frequently in clinical practice because of their broad spectrum activity, low toxicity and resistance to the bacterial β-lactamases induced in bacterial population. Ceftizoxime inhibits a wide variety of aerobic/anaerobic gram positive and gram negative bacteria. Pharmacokinetics of ceftizoxime was studied in healthy and hyperthermic condition in sheep [(3)] and in acute renal failure condition in goat [(4)]. Pharmacokinetics of ceftriaxone in mastitic condition was studied in goats [(5)]. But no study was done that explore the bioavailability and pharmacokinetics of ceftizoxime in mastitic goat. Considering the above the present research work was undertaken with the objectives to explore pharmacokinetics of ceftizoxime in healthy lactating and induced mastitic goats after intravenous administration.

Materials and Methods

Chemicals

Ceftizoxime (technical grade, purity ≥ 90%) was used as the test drug which was obtained from M/s Alembic Ltd, Vadodara, India. All the other chemicals were obtained from E. Merck (India) and Sigma Chemicals Co., USA

Animals

Clinically healthy lactating black Bengal female goats weighing between 10-12 kg of approximately 1½ - 2 year age were used in this experiment. The animals were caged individually in custom's made metabolic cage (stainless steel). The temperature of the experimental animal room was maintained at 22^0C (± 3^0C). Artificial lighting facilities were also provided. The animals were stall fed and water was provided *ad libitum*. The composition of the feed was 2 parts wheat husk, 1 part groundnut cake, 1 part crushed gram, 1 part crushed maize and 2 parts green. The animals were dewormed with a single oral dose of albendazole at 10mg/kg body weight 30 days prior to the onset of study. Before the start of the experiment, the animals were acclimatized for 7 days.

The research project was approved by Institutional Animal Ethics Committee.

Design of experiment

12 goats were divided into 2 groups i.e healthy and mastitic consisting of 6 goats in each. Disposition kinetics of ceftizoxime was carried out following intravenous administration at the dose rate of 10 mg/kg dissolved in 2ml of distilled water in both the groups.

Collection of samples

Blood samples (2 ml) were collected from the jugular vein in heparinized test tubes at '0' (prior to drug administration) and at 0.08, 0.16, 0.33, 0.50, 1, 2, 4, 6, 8, 12, 24, 36 and 48 hr post drug administration (pd). Plasma was then separated by centrifugation at 3000 rpm for 20 min and (0.50 ml) was utilized for the analysis of ceftizoxime concentration.

Milk samples (1 ml) were collected from both teats into the test tubes at '0' (prior to drug administration) and at 0.50, 1, 6, 12, 24, 48, 72, 96, 120 and 144 hr pd. Milk samples from both the teats were mixed and 0.50 ml was taken for estimation of drug concentration. All the samples were stored at refrigerated condition (-20^{0}C) till further use. The remaining milk sample was utilized for estimation of some enzymatic activity.

Isolation and identification of pathogenic bacterial strains and MICs determination

Milk samples were collected from cows with clinical mastitis. The isolation and identification of bacterial strains were performed using standard bacteriological methodology. The strains were stored at -30^{0}C until used. When needed, the strains of *Escherichia coli* were grown on tryptose agar. The strain isolated from clinical mastitis was nonhaemolytic, intermediately serum resistant and sensitive to ceftizoxime in vitro. Determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were performed by using a stock solution of ceftizoxime at 100 µg/ml. [6]

Induction of mastitis: Mastitis was induced by using pathogenic strain of Escherichia coli as stated before according to the method of our laboratory Sar *et al.* (2006). [5]

Biomarkers for diagnosis of mastitis

(i) *Bromo thymol blue paper test (BTB)*

This test is based on the fact that milk from infected udders (mastitis) is usually alkaline in reaction (pH 7 to pH 7.4) and this condition can be detected by observing the color change shown by a suitable pH indicator added to milk.

(ii) *Catalase activity in milk*

Estimation of catalase activity in milk was performed by standard procedure. [7]

(iii) *Lactoperioxidase activity in milk*

Estimation of lactoperoxidase activity in milk was conducted by standard procedure. [8]

Analysis of ceftizoxime

The concentration of ceftizoxime in plasma and milk was estimated by standard procedure of our laboratory [5, 9]. A SHIMADZU LC-20AT liquid chromatograph coupled with Diode-Array detector (UV-VIS) attached with computer SPD MXA 10 software was used for the detection. Mobile phase consists of Glacial acetic acid: Acetonitrile: water (5:20:75) having a final pH of 2.1. A 5µLuna C18 (2); 250 x 4.6 mm(RP) column was

used with the flow rate and wavelength of 1ml/min and 254 nm respectively. 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 by a standard stock solution of 100 ppm of analytical grade of ceftizoxime prepared in distilled water. The retention time of ceftizoxime was found to be 3.46 min.

Recovery of ceftizoxime from plasma/milk

Recovery of ceftizoxime from goat plasma/milk was carried out *in vitro* after fortifying with 0.25, 0.50, 1, 10, 20, 40, 50 and 100 µg/ml of Ceftizoxime in plasma /milk. The sample drug analysis was done by HPLC. The recovery percentage ranges from 90-95. The limit of detection of ceftizoxime was 0.25 µg ml^{-1} in plasma/ milk.

Pharmacokinetic parameters

Pharmacokinetic parameters of ceftizoxime were determined from computerized curve fitting programme 'PHARMKIT' supplied by the Department of Pharmacology, JIPMER, Pondicherry, India and from standard formula [10]. Dosage regimen was designed by standard formula of Rowland and Tozer.[11]

Statistical analysis

The results were expressed as Mean ± Standard error (S.E.). The data were analyzed statistically by students't' test using SPSS 10.0 version of software.

Results

Antibiotic sensitivity and induction of mastitis

The MIC and MBC of ceftizoxime against the study strain of *Escherichia coli* at a concentration of 40×10^6 c.f.u/ml is 1.25 and 2.50 µg/ml respectively. Mastitis was induced by using 40 colony flocculating unit (CFU) of *E. coli.* After 24 hr, all goats developed clinical signs and symptoms of mastitis such as fever, tachycardia, anorexia, depression, severe swelling and pain in the udder and serous like milk along with changes in biomarkers for mastitis like bromo thymol blue test, lactoperoxidase and milk catalase activity etc.

Disposition kinetics of ceftizoxime in healthy lactating and mastitic goats in intravenous administration

Plasma level and kinetics

The maximum mean plasma concentrations of ceftizoxime were found to be 83.43 ± 3.39 and 90.83± 6.41 µg ml^{-1} for healthy lactating and mastitic goats at 0.08 hr, followed by a sharp decline and the minimum plasma concentrations of 1.28 ± 0.07 µg ml^{-1} was found at 24 hr pd in healthy lactating and 0.91±0.01µg ml^{-1} at 72 hr pd in mastitic goats [Figure 1]. Pharmacokinetic parameters [Table 1] showed that mean values of β and t½ β were respectively 0.12 ± 0.003 hr^{-1} and 6.24±0.18 hr in healthy and respectively 0.05± 0.002 hr^{-1} and 14.95 ± 0.65 hr in mastitic goats. Both the values of β

and t½ β were decreased and increased significantly (P< 0.01) in mastitic goats compared to healthy goats. Mean AUC value was increased significantly (P<0.01) in mastitic goats compared to healthy lactating goats. On the other hand, significantly increased and decreased Vd_{area} and Cl_B value in healthy lactating goats compared to mastitic goats were observed. The value of $K_{21,}$ $K_{12,}$ Vd_C ,Vd_{SS} and f_C values were not significantly altered in both the groups. But the value of K_{el} was decreased significantly (P<0.01) in mastitic goat. Mean MRT value in mastitic goats increased significantly (P<0.01) compared to healthy lactating goats.

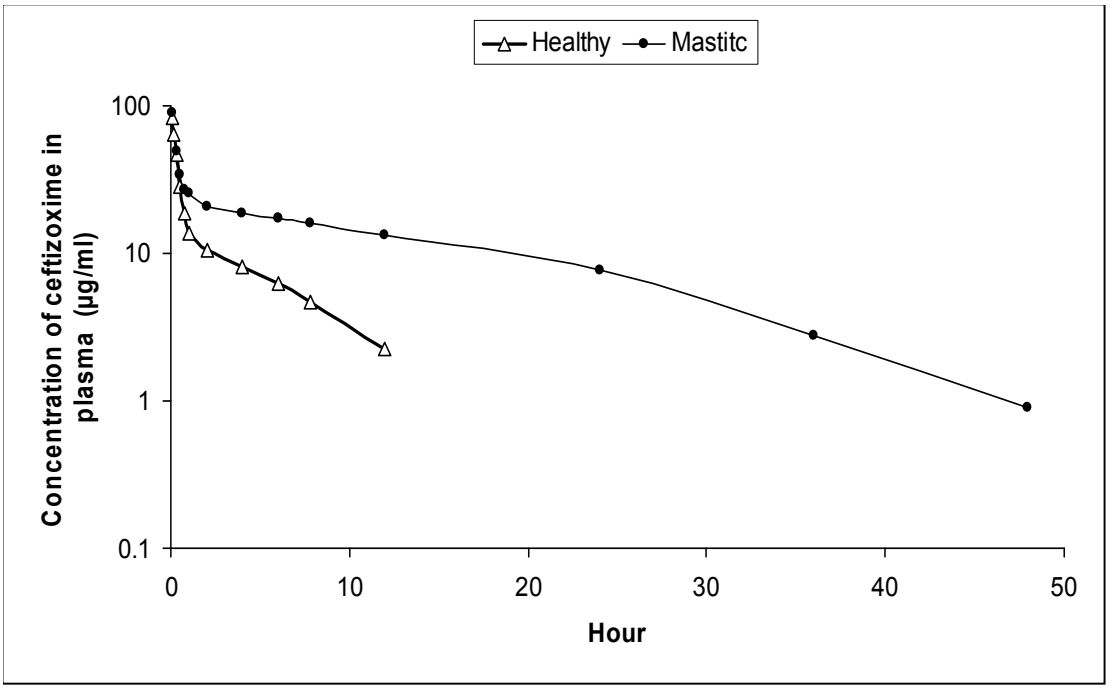

Figure 1: Semilogarithmic plot of mean plasma concentration of ceftizoxime in healthy lactating and mastitic goats after single dose intravenous administration at 10 mg kg^{-1} body weight

Table 1: Mean kinetic parameters of ceftizoxime in healthy lactating and mastitic goats after single dose intravenous administration at 10 mg kg^{-1} body weight

(Mean of 6 replicates with SE)

Kinetic parameters	Healthy lactating	Mastitic
Cmax(μg ml^{-1})	88.78±5.42	90.83±6.41
C^0_P (μg ml^{-1})	109.48±5.36	117.30 ± 11.88
α (hr^{-1})	4.12±0.55	4.00±0.57
$t_{½}α$ (hr)	0.12± 0.03	0.19±0.02
β (hr^{-1})	0.12 ± 0.003	0.05**±.002
$t_{½}β$ (hr)	6.24±0.18	14.95**±0.65
AUC (μg hr ml^{-1})	185.22 ± 7.69	574.06** ± 38.93
Vdarea (L kg^{-1})	0.49 ± 0.02	0.39*± 0.03
Vd_C (L kg^{-1})	0.09±0.004	0.09 ± 0.001
Vd_{SS} (L kg^{-1})	0.45±0.01	0.39 ± 0.03
Cl_B (L kg^{-1} hr^{-1})	0.05±0.002	0.02 **± 0.001
MRT(hr)	7.58±0.21	20.31**±0.86
K_{12} (hr^{-1})	3.06±0.42	2.93± 0.47
K_{21} (hr^{-1})	0.76±0.08	0.89 ± 0.09
K_{el} (hr^{-1})	0.59±0.03	0.21** ± 0.02
f_C	0.19±0.01	0.23 ± 0.01
T ~P	4.67±0.24	3.41* ± 0.09

*P <0.05, **P <0.01 compared to healthy lactating goats.

Milk level

Milk concentration of ceftizoxime [Figure 2] started to increase from 0.5 hr (2.80 ± 0.40μg ml^{-1}) , achieved its peak level at 12 hr (17.74 ± 1.51 μg ml^{-1}) followed by a slow decline in concentration and came to its minimum concentration of 0.59 ± 0.08 μg ml^{-1} at 96 hr in healthy lactating goats. On the other hand, Ceftizoxime achieved a concentration of 2.35 ± 0.44 μg ml^{-1}at 0.5 hr which started to increase with a peak level of 40.98 ± 3.82 μg ml^{-1} at 24 hr and persisted upto 144 hr pd with a minimum concentration of 0.43±0.04 μg ml^{-1} in milk of mastitic goats.

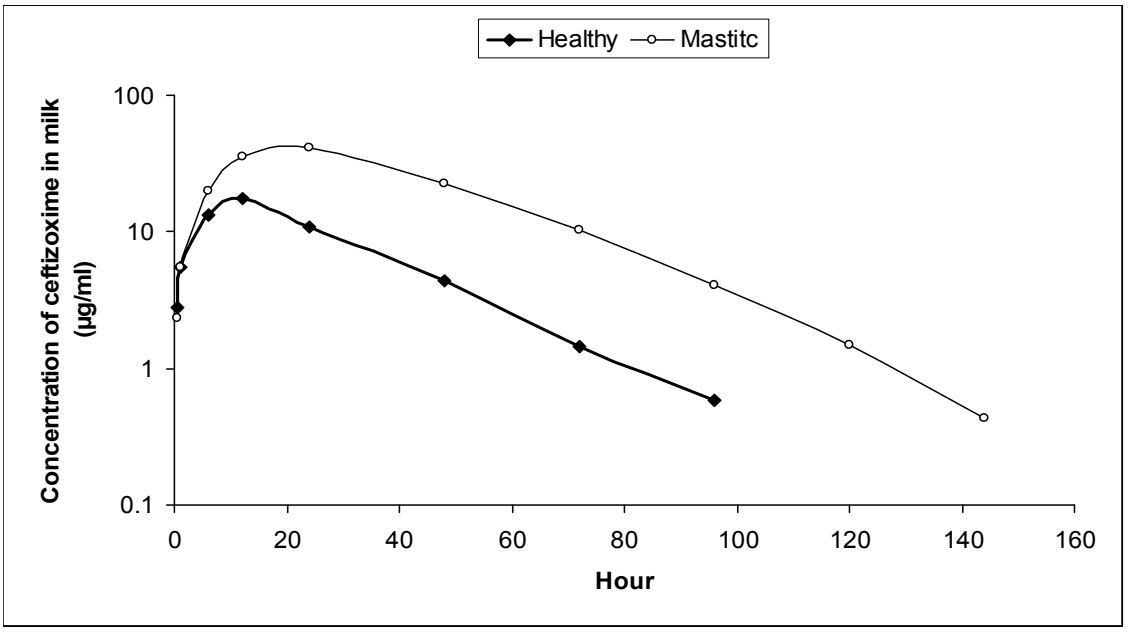

Figure 2: Semilogarithmic plot of mean milk concentration of ceftizoxime in healthy lactating and mastitic goats after single dose intravenous administration ceftizoxime of at 10 mg kg^{-1} body weight

Bromo thymol blue paper test

A greenish blue or blue color is developed in milk collected from mastitic animals. But Bromo thymol blue paper test gave negative result at 120 hrs of collected milk samples.

Effect of Ceftizoxime on milk enzymes

Catalase activity [Table 2] and lactoperoxidase activity [Table 3] were increased significantly ($P<0.05$) after induction of mastitis. But, at 120hr of single dose intravenous administrations of ceftizoxime at 10 mg kg^{-1} both the enzyme activities reached to a value which was nearer to the values before the induction of mastitis.

Table 2: Mean milk catalase activity (μ mole H_2O_2 hydrolysed min^{-1} ml^{-1}) in mastitic goats after single dose intravenous administration of ceftizoxime at 10 mg kg^{-1} body weight

(Mean of 6 replicates with SE)

	Mean milk catalase activity (μ mole H_2O_2 hydrolyzed min^{-1} ml^{-1} of milk)
Control before mastitis	30.26± 3.00
Induction of mastitis	52.85* ± 9.63
Ceftizoxime administration	
Time (hr)	
24	40.85*±1.21
48	38.56*±0.98
72	35.28±2.32
96	34.85±1.86
120	32.26± 3.59

*$P<0.05$ compared to control before mastitis

Dosage regimen for ceftizoxime:

Based on the relevant pharmacokinetics parameters derived from the respective plasma concentration time profile, rational dosage regimens for ceftizoxime both for healthy and mastitic goats have been formulated and presented in table 4.

Clinical efficacy of the drug

Mastitis was clinically cured after 5 days pd. Sign and symptom of mastitis such as anorexia, hyperthermia, inflammed udder, milk turbidity etc. were disappeared. Bromo thymol blue paper test gave negative result. Catalase and lactoperoxidase activity were normalized after 5 days [Table 2, 3].

Table 3: Mean milk lactoperoxidase activity (mole min^{-1} L^{-1}) in mastitic goats after single dose intravenous administration of ceftizoxime at 10 mg kg^{-1} body weight

(Mean of 6 replicates with SE)

	Mean milk lactoperoxidase activity (mole min^{-1} L^{-1})
Control before mastitis	29.45± 0.58
Induction of mastitis	17.20 *± 0.45
Ceftizoxime administration	
Time (hr)	
24 hr	18.85± 1.21
48hr	22.56± 0.98
72hr	24.28± 2.32
96hr	26.85± 1.86
120hr	30.25± 0.45

*P<0.05 compared to control before mastitis

Table 4: Intravenous dosage regimen of Ceftizoxime in Goats

Group	**D_L (mg kg^{-1})**	**D_M (mg kg^{-1})**	**C_{max} (µg ml^{-1})**	**C_{min} (µg ml^{-1})**	**Dosing interval (hr)**
Healthy	7.42	4.9	15.31	5	9.85≅ 10
Mastitic	5.9	3.9	15.6	5	23.66≅ 24

DL = Loading Dose, DM = Maintenance Dose

Cmax = Maximum therapeutic concentration

Cmin = Minimum therapeutic concentration

Discussion

Plasma ceftizoxime concentrations were non significantly higher from 0.08 to 1 hr and significantly higher from 2 to 24 hr at different time in mastitic goats compared to lactating goats. Previous work of ceftizoxime on goat reported maximum mean plasma concentration of 78.85 ± μg ml^{-1} for Ceftizoxime in healthy goats [4] which are nearer to the value of present study, whilst a higher concentration of Ceftizoxime in dog and cat was reported[12] which might be due to species variation. Semi logarithmic plot of mean plasma level time profile of Ceftizoxime in both the groups suggest "two compartment open model." [4, 12] Febrile condition induces acidosis and sodium chloride retention consequent upon occurrence of oliguria and certain biochemical changes in animals [13]. In the present study, fever was developed during acute mastitis. It is expected that during fever the urinary pH will tend to decrease and there will be greater unionization rate of acidic drug ceftizoxime resulting in more reabsorption of the drug from DCT of nephron in mastitic goats leading to significantly lower K_{el} value. Further, greater unionization of ceftizoxime in systemic acidosis may have led to a consistent overall higher level of the drug associated with prolonged t½ β values along with higher AUC and MRT values in mastitic goats.

Milk concentration of Ceftizoxime in mastitic goats was significantly higher compared to healthy lactating goats from 6 hr onwards [5]. The passage of most antimicrobial agents from the systematic circulation into milk is in accordance with the pH partition hypothesis. A change in the pH of milk will influence the drug level produced in the milk. For weak organic bases, the milk to plasma concentration ratio of total drug will decrease with increasing pH of milk [10]. Conversely, higher milk level of weak acidic antibiotic like Ceftizoxime were attained in goats affected with mastitis (milk pH reaction may be increased up to 0.7 of or a pH unit) than in healthy lactating goats.

Mean milk lactoperoxidase activity decreased in mastitic goats which is a natural antimicrobial system of milk. Lactoperoxidase does not have sufficient substrate (thiocyanate, hydrogen peroxide) to produce an effective antibacterial system in milk as one of the potential causes of failure of endogenous antibacterial factors to operate effectively [14]. The increased activity of catalase in milk suggests decreased level of hydrogen peroxide, a reactive oxygen species leading to stabilization of cellular membrane of mammary gland. After intravenous administration of ceftizoxime, mean catalase and lactoperoxidase activity reached to the level of control value (before mastitis). It indicates the clinical efficacy of Ceftizoxime against mastitis.

It has been reported that the sensitive organisms are inhibited within the concentration range of 5 to 15 μg ml^{-1}. So the calculated dosage regimens [Table 4] would be expected to be effective in combating mastitis in goats.

Conclusion

From the above study, it can be concluded that diseased condition like mastitis alter significantly the disposition kinetics of ceftizoxime in healthy goats after intravenous administration. It would be further mentioned that the dose and dosing interval of ceftizoxime should be reduced and increased respectively in mastitic goats compared to healthy goats.

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