Pharmacokinetics of Ceftriaxone in Healthy and Mastitic Goats With Special Reference to Its Interaction With Polyherbal Drug (Fibrosin®)

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ABSTRACT

The pharmacokinetic profile of ceftriaxone was studied in healthy lactating goats, healthy lactating goats treated with single oral administration of a polyherbal drug Fibrosin®, and induced mastitic goats with a pre-single-dose oral administration of Fibrosin after a single intravenous dose at 50 mg/kg. Ceftriaxone was present for 1 hour in plasma of healthy lactating goats; the kinetic behavior followed the "1-compartment open" model while it persisted for 2 hours and 0.50 hour in plasma of healthy lactating goats and mastitic goats with 1 hour pre-single dose oral administration of Fibrosin, respectively. The kinetic behavior of ceftriaxone followed the "1-compartment open" model in mastitic goats even in the presence of Fibrosin, while it followed the "2-compartment open" model in Fibrosin-treated healthy goats. Biological half-life ($t_{½}$ β) of ceftriaxone (0.10 ± 0.01 h) in mastitic goats in presence of Fibrosin decreased significantly compared to $t_{½}$ β (0.21 ± 0.01 h) in healthy lactating goats. Ceftizoxime, a major active metabolite of ceftriaxone, persisted for a longer period in milk after single-dose intravenous administration of ceftriaxone. Ceftizoxime was also available up to 72 hours post-dosing in urine of healthy lactating goats, while ceftriaxone was recovered at 24 hours followed by recovery of its metabolite ceftizoxime at 48 and 72 hours post-dosing in urine of Fibrosin-treated healthy and mastitic goats, respectively. Ceftriaxone induced microsomal cytochrome P450 in liver of healthy goats after intravenous administration.

INTRODUCTION

Mastitis is an inflammatory condition of the mammary gland irrespective of causes. It is a global problem in livestock. It is characterized by physical, chemical, and microbiological changes in the milk and pathological changes in glandular tissues of the mammary gland. Ceftriaxone, a third-generation

cephalosporin, is being used more frequently in clinical practice because of its broad spectrum of activity, low toxicity, and resistance to the bacterial β-lactamases induced in the bacterial population. But ceftriaxone is still an experimental drug in veterinary clinical practice. In the present study, the pharmacokinetics of ceftriaxone with its metabolic pathway, the protective effect of ceftriaxone in mastitis of goats and its interaction with polyherbal drug (Fibrosin®) were evaluated.

MATERIALS AND METHODS

Drugs

The drugs used in this study were ceftriaxone (Aristo Pharmaceuticals, India), ceftizoxime (GlaxoSmithKline Pharmaceuticals Ltd., Nashik, India) and Fibrosin® (Legend Remedies Pvt. Ltd., India). Fibrosin is a polyherbal drug marketed by Legend Remedies for concomitant therapy with antibiotics in clinical and sub-clinical mastitis. The company's literature reported that it aids in the cleansing of udder tissues and down flow of milk.[1]

Composition of Fibrosin

Kanchanar-gugal	Chitrak-mula	Punar-navastaka	Trifala (Myrobalan + Black Myrobalan + Emblic)-gugal	Apamarga
Scientific name of Kanchan: *Bauhinia veriegata linn.*	Scientific name: *Plumbago zeylanica.*	Scientific name: *Triaanthema monogyna*	Scientific name of Myrobalan: *Terminalia belerica Retz.* + Scientific name of Black Myrobalan: *Terminalia chebula Retz.* + Scientific name of Emblic: *Phylanthus amblica*	Scientific name: *Achyranthes aspera linn*

Chemical Compositions of the Medicinal Plants

Kanchan	Chitrak-mula	Punar-navastaka	Trifala	Apamarga
Gummy materials, tannin, sterols	**Root/bark** Plumbegin, free glucose and fructose (2.7%), enzymes (protease and invertase) **Leaves and stem** Volatile oil **Flowers** Azulein (5-methoxy quercetin 3-rhamnoside), 3 rhamnosides of delphinidin	Alkaloid (punarnavine), other unidentified bases, fatty alcohol, terols (β-itosterol, α-sitosterol)	**a) Myrobalan fruit** Tannin, β-sitosterol, gallic acid, ellagic acid, ethyal gallate, galloyl glucose, chebulagic acid, mannitol, glucose, galactose, fructose, rhamnose **b) Black Myrobalan fruit** Tannin, polyphenolic compounds (chebulinic acid, chebulagic acid, gallic acid, corilagin, number of unidentified phenolic constituents), anthraquinone dye stuff **c) Emblic fruit** Vitamin viz., ascorbic acid, amino acid viz., glycine, tannin, polyphenolic compounds (viz., corilagin, ellagic acid, terchebin, gallic acid, chebulic acid, chebulagic acid, chebulinic acid), fixed oil, lipids viz., phosphatides, essential oil	Pungent oil, sterols viz., β-sitosterol and γ-sitosterol, terpenoid constituents

Animals

Clinically healthy lactating black Bengal goats (1.5 to 2 years of age) weighing 10-12 kg were considered for this study. A total of 21 goats were included in this study out of which 18 goats were divided into 3 groups consisting of 6 goats in each group and considered for pharmacokinetics study of ceftriaxone. Other 3 goats were used for microsomal study to determine cytochrome P450 content. The animals were caged individually in custom-made, stainless steel cages. The temperature of the experimental animal room was maintained at 22°C (±3°C). 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 graham, 1 part crushed maize, and 2 parts greens. The animals were dewormed with a single oral dose of rafoxanide powder (USV Limited, Mumbai, India) at 7.5 mg/kg body weight 30 days prior to the onset of study. Before the start of the experiment, the animals were acclimatized for 7 days. During this period, body temperature, volume of urine and quantity of feces excreted, and any other abnormalities were recorded daily.

Assay Procedures

Ceftriaxone and ceftizoxime concentrations were measured by computerized double-beam UV-VIS spectrophotometry and high-performance liquid chromatography (HPLC).

Spectrophotometric Assay

The absorbance of plasma supernatant was read in computerized double-beam UV-VIS spectrophotometers at 272 nm wavelength for ceftriaxone and at 254 nm for ceftizoxime against a blank prepared with plasma collected at 0 hour. The absorbance of milk supernatant was read at 270 nm for ceftriaxone and 260 nm for ceftizoxime against a blank prepared with milk collected at 0 hour. The absorbance of urine supernatant was read at 260 nm for ceftriaxone against a blank prepared with urine collected at 0 hour. Ceftizoxime concentration in urine could not be detected by spectrophotometric assay.

Plasma/milk/urine sampling

The following were added to a centrifuge tube containing 0.50 mL of plasma/milk/urine: 4 mL (for plasma), 6 mL (for milk), or 10 mL (for urine) saturated ammonium sulphate in 2.5% sulfuric acid (H_2SO_4, 98%) and formic acid (90%); and 0.05 mL (for plasma), 0.08 mL (for milk), or 0.12 mL (for urine). The tubes were shaken vigorously for 1 minute. The whole aliquot was centrifuged at 5000 rpm for 20 minutes. The supernatant was collected after passing through a filter paper (Whatman No.1). Concentration of ceftriaxone and ceftizoxime was then calculated from standard curve prepared earlier and expressed as µg/mL. Recovery of ceftriaxone from plasma/milk/urine and ceftizoxime from plasma/milk was carried out in vitro to ascertain the reliability of analytical method after fortifying with 1, 2, 4, 10, 20, 30, 40, and 50 µg/mL of ceftriaxone and ceftizoxime in different substrates. The absorbencies against several concentrations of ceftriaxone and ceftizoxime at their respective wavelengths were plotted on graph paper, and linearity was found to be maintained. The recovery was within a range of 82.55% to 93.05% and therefore the analytical method was considered for estimation of ceftriaxone/ceftizoxime in this experiment. The sensitivity of the method for both ceftriaxone and ceftizoxime was 0.90 µg/mL and the limit of detection was 1 µg/mL.

Chromatographic Assay

Analysis by HPLC was performed on a Hewlett Packard model 1050 liquid chromatograph coupled with a variable wavelength UV-VIS detector attached with a 3392 A integrator equipped with a Hamilton syringe having a 25 µL loop and an RPC-18 cartridge column. For the mobile phase, tetraheptyl ammonium bromide (3.2 g) was dissolved in 400 mL of acetonitrile; 44 mL of buffers pH 7.0 and 4 mL of buffer pH 5.0 were added. Water was then added to make 1000 mL. The whole mixture was filtered through a membrane filter of 0.5 µm and degassed. Buffers (pH 7.0 and pH 5.0) and the mobile phase were prepared according to procedure mentioned in USP. The flow rate was maintained at 1.5 mL/min. The maximum absorbance was at 280 nm (HPLC assay).

Plasma sampling

Acetonitrile (1 mL) was added to a centrifuge tube containing 1 mL of plasma and was shaken vigorously for 1 minute. The whole aliquot was centrifuged at 5000 rpm for 20 minutes. The supernatant was collected after passing though a filter paper (Whatman No. 1). The clear supernatant was reserved, and 20 µL of this was injected for HPLC assay.

Milk sampling

To 2 separate centrifuge tubes containing 1 mL of milk, 4 mL of mobile phase to the first one and 1 mL of acetonitrile to the other one were added and shaken vigorously for 1 minute. The whole aliquot was centrifuged at 5000 rpm for 20 minutes. The supernatant was collected after passing through a filter paper (Whatman No. 1). The

clear supernatant was reserved, and 20 µL of this was injected for HPLC assay.

Urine sampling

To 2 separate centrifuge tubes containing 1 mL of urine, 4 mL of mobile phase to the first one and 1 mL of acetonitrile to the other one were added and shaken vigorously for 1 minute. The whole aliquot was centrifuged at 5000 rpm for 20 minutes. The supernatant was collected after passing through a filter paper (Whatman No. 1). The clear supernatant was reserved, and 20 µL of this was injected for HPLC assay.

Stock solution of ceftriaxone and ceftizoxime (100 ppm) and mixture of both (50 ppm, 50 ppm) were prepared in water as standards. The retention times of ceftriaxone and ceftizoxime were 2.40 and 1.11 minutes, respectively (Figure 1) for both the standards prepared in water. The retention times of the ceftriaxone and ceftizoxime occurring in plasma/milk/urine were compared with that of the external standard and the data were recorded in an HP3392 A integrator. The recovery of ceftriaxone was 75%-80%) from plasma, 94.55% from urine, and 30% from milk using acetonitrile whereas it was much less when mobile phase was used. The recovery of ceftizoxime was 92.25% from urine, 30.40% from milk by using mobile phase, but was much less when acetonitrile was used. The linearity for 2 compounds was verified by calibration curve. The recovery of ceftriaxone was greater in acetonitrile while the recovery of ceftizoxime was greater in mobile phase. Recovery from plasma and milk by HPLC for both the compounds was much less than with spectrophotometric assay. Therefore, both ceftriaxone and ceftizoxime in plasma and milk were estimated by UV spectrophotometer only, while the HPLC method was used for estimation and identification of ceftizoxime in urine sample. Additionally, HPLC was used for identification of ceftriaxone and or ceftizoxime in plasma, milk and urine (Figure 2).

Figure 1. Chromatograms of (a) ceftriaxone (analytical grade: 100 ppm), (b) ceftizoxime (analytical grade: 100 ppm), (c) ceftriaxone and ceftizoxime (50 ppm, 50 ppm) in water, and (d) ceftriaxone and ceftizoxime (50 ppm, 50 ppm) in mobile phase.

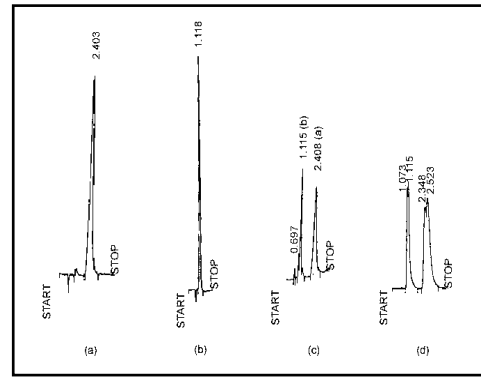
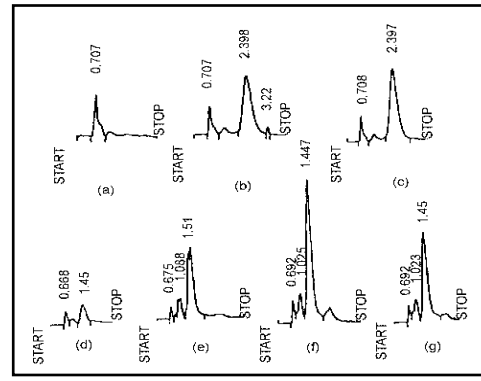


Figure 2. Chromatograms of (a) control plasma and ceftriaxone and/or ceftizoxime extracted from plasma ((b), (c)), and (d) control milk and ceftriaxone and/or ceftizoxime extracted from milk ((e), (f), (g)).



Assay of milk enzyme activity

Milk enzyme activity, including that of alkaline phosphatase, catalase, and lactoperoxidase, and reduced glutathione levels in milk were studied in healthy and mastitic goats. Estimation of alkaline phosphatase activity in milk was done according to the method described by Bernt.[2] Estimation of catalase activity was done according to the method described by Maehly and Chance.[3] Reduced glutathione levels in milk was estimated according to the method described in *Methods of Enzymology*.[4] Lactoperoxidase activity was determined according to the method described by Makinene and Tenovuo.[5]

Microsomal Study

Cytochrome P450 content in the liver was estimated after administration of Fibrosin alone, Fibrosin and ceftriaxone in combination, and ceftriaxone alone. A half bolus of Fibrosin (1.9 g) was administered orally to all the healthy goats. The animals were slaughtered after 48 hours and each liver was collected for estimation of cytochrome P450 content. A single dose of ceftriaxone was administered intravenously to the healthy goats at 50 mg/kg body weight. A half bolus of Fibrosin (1.9 g) was administered orally 1 hour before ceftriaxone administration to each healthy goat. The animals were slaughtered after 48 hours and cytochrome P450 content was estimated in the liver. A single dose of ceftriaxone was administered intravenously to the healthy goats at 50 mg/kg body weight. The animals were slaughtered after 48 hours for determination of cytochrome P450 content.

Measurement of cytochrome P450 contents

Cytochrome P450 contents of the microsomal pellet in liver of all animals were estimated according to the methods described by Omura and Sato.[6] Microsomal protein was measured according to the method described by Lowry et al.[7]

Induction of mastitis

Slant culture of coagulase-positive Staphylococci obtained from the Department of Veterinary Microbiology, West Bengal University of Animal and Fishery Sciences, was reserved in Tryptic Soya (T_S) broth and incubated for 24 hours at 37°C. Immediately after incubation, the subculture was again reserved in T_S broth and kept at 37°C for 18 h. This subculture was preserved at 4°C. Taking 0.20 mL of this broth subculture, serial dilution was performed in 9 sugar tubes containing 1.80 mL of phosphate buffer saline (PBS; pH 7.4) under strict aseptic condition. Broth subculture (100 µL) from sugar tubes having dilution of 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9} were poured and spread on plates containing mannitol salt agar. These 4 plates were kept at 37°C for 24 hours and the colony numbers were counted. Plates having broth culture of 10^{-7} dilution showed 15 c.f.u. The serial dilution process was again repeated by taking the original broth culture preserved at 4°C up to 10^{-6} dilution. One mL of this diluted subculture was taken and 4 mL of PBS was added to it. This 5 mL of subculture was administered intracisternally to the right quarter of lactating goats. After 24 hours, animals showed the symptoms of slight fever, red colored urine, and swelling and pain at both the quarters on touching. These symptoms waned after 4 hours. One mL of broth subculture preserved at 4°C was again taken and serial dilution was made in test tubes containing 9 mL of nutrient broth; the dilution was made up to 10^{-5} aseptically. Broth subculture (100 µL) of different dilutions was poured and spread in mannitol salt agar plates. A dilution of 10^{-5} showed 35 c.f.u. The dilution was again repeated up to 10^{-3} dilution from which 1 mL was taken and introduced intracisternally to the right quarter of the experimental goats. A confirmatory test was performed after inoculation of organisms using BTB (Bromo thymol blue) paper test at 24-hour intervals; the colors observed in the paper have been presented in Table 1.

Table 1. Color Changes of BTB Paper at 24-hr Intervals After Inoculation of Coagulase (+ve) Staphylococci.

	Color of Milk	
Time (h)	**Left Quarter**	**Right Quarter**
24	Light green	Light green
48	Light green	Bit darker green
72	Bit darker green	Dark deep green
96	Dark green	Deep bluish green
120	Greenish purple	Greenish purple

Condition of the induced mastitic animals

After 120 hours of inoculation, both the quarters of the induced mastitic goats were swollen, hot, and hard, though the right quarter was harder than left quarter. The

goats showed signs of pain at both the quarters on touching. The goats also showed agalactia and defecated semisolid feces with a slight increase of temperature from 39.66°C to 39.88°C.

Dosing

A single dose of ceftriaxone was administered intravenously to 3 different groups containing 6 goats each. A single dose of ceftriaxone at 50 mg/kg dissolved in 5 mL of distilled water was administered to each healthy lactating goat through the jugular vein. To another group of healthy lactating goats, a single dose of ceftriaxone dissolved in 5 mL of distilled water was administered through the jugular vein at 50 mg/kg with a 1-hour pre-single-dose oral administration of Fibrosin (1.9 g). To experimentally induced mastitic goats, a single dose of ceftriaxone dissolved in 5 mL of distilled water was administered to each animal through the jugular vein at 50 mg/kg with a 1-hour pre-single-dose oral administration of Fibrosin (1.9 g).

Collection of Samples

Blood samples were collected at 0 and at 0.08, 0.16, 0.25, 0.33, 0.50, 0.66, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 60, 84, 96, and 120 hours post-dosing. Plasma was then separated by centrifugation at 3000 rpm for 20 minutes and 0.50 mL was utilized for the analysis of ceftriaxone and or ceftizoxime concentration.

Milk samples (1 mL each) were collected from both teats into the test tubes at 0 and at 0.08, 0.16, 0.25, 0.33, 0.50, 0.66, 1, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, 240, 288, 336, 360, and 480 hours post-dosing. Additional milk samples from healthy lactating goats without Fibrosin treatment were collected at 576, 600, and 720 hours post-dosing. A sufficient amount of milk samples could not be collected from induced mastitic goats from 0.08 hour to 6 hours due to agalactia. Urine samples were collected at 0, 24, 48, and 72 hours post-dosing, keeping the experimental animals in the stainless steel cages.

Pharmacokinetic and Statistical Analysis

Pharmacokinetic parameters of ceftriaxone were determined from the computerized curve-fitting program "PHARMKIT" supplied by the Department of Pharmacology, JIPMER, Pondicherry, India. Mean values, standard error, and analysis of variance of the tabulated data were calculated where applicable using the statistical software program M Stat.

Identification of Metabolites in Plasma, Milk, and Urine

Plasma, milk, and urine samples of healthy and induced mastitic goats and milk samples of mastitic cows were examined by spectrophotometer and HPLC under the aforementioned operating conditions.

RESULTS

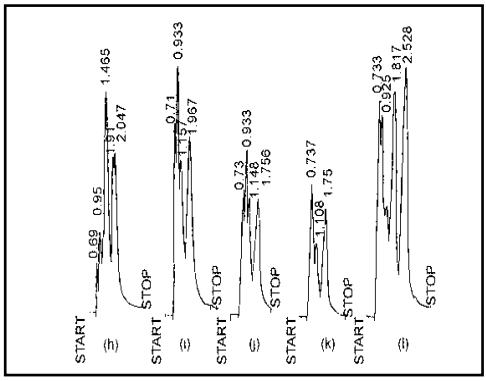
Milk Enzyme

Mean normal alkaline phosphatase activity in milk of healthy goats ranged between 4920.00 ± 1062.35 and 7840.00 ± 499.42 n mole PNP produced/hr/mL. Normal mean milk catalase activity in healthy goats ranged from 16.00 ± 0.86 to 27.76 ± 3.00 μ mole H_2O_2 hydrolyzed/min/mL and reduced glutathione level in milk of healthy goats was within a range of 319.90 ± 15.99 to 600.00 ± 51.96 n mole GSH/mL. Mean milk alkaline phosphatase activity increased significantly ($P < 0.05$) to 12160.00 ± 831.40 n mole PNP produced/hr/mL of milk in mastitic goats. Catalase activity in milk also increased significantly ($P < 0.05$) to 66.94 ± 9.63 μ mole H_2O_2 hydrolyzed/min/mL in mastitic goats, but mean reduced glutathione level was not altered significantly. Mean lactoperoxidase activity (15200.00 ± 4541.91 μ mole/min/L) in mastitic goats was decreased compared with 27400.00 ± 5860.27 μ mole/min/L of healthy goats.

Chromatograms of Metabolites

Chromatograms of identified metabolite in milk and urine are presented in Figures 2 and 3.

Figure 3. Chromatogram of (h) control urine and ceftriaxone or ceftizoxime extracted from urine ((i), (j), (k), (l)).



Plasma Concentration

The plasma concentrations of ceftriaxone in healthy lactating, Fibrosin-treated healthy lactating, and Fibrosin-treated induced mastitic goats are shown in Table 2. Maximum plasma concentration of ceftriaxone was achieved at 0.08 hour, (135.00 ± 4.35 μg/mL) followed by sharp decline, and the minimum plasma concentration was recorded at 1 hour post-dosing (7.03 ± 1.54 μg/mL) in healthy lactating goats without Fibrosin after single-dose intravenous administration of ceftriaxone at 50 mg/kg. Ceftriaxone achieved its peak plasma concentration of 230.00 ± 11.54 μg/mL at 0.08 hour, followed by gradual decline, and reached its minimum plasma concentration of 16.60 ± 1.44 μg/mL at 2 hours post-dosing in healthy lactating goats with 1-hour pre-single-dose oral administration of Fibrosin. Ceftriaxone persisted at its maximum plasma concentration of 141.00 ± 14.22 μg/mL at 0.08 hour, followed by decline in concentration at a faster rate and reached its minimum plasma concentration of 9.46 ± 3.26 μg/mL at 0.50 hour post-dosing in mastitic goats with 1-hour pre-single-dose oral administration of Fibrosin. The plasma concentration of ceftriaxone increased significantly ($P < 0.05$) in Fibrosin-treated healthy lactating goats compared with healthy and mastitic goats starting from 0.08 hour to 0.33 hour post-dosing. Ceftriaxone persisted for 2 hours in Fibrosin-treated healthy lactating goats but was present for only 1 hour in healthy goats without Fibrosin and 0.50 hour in mastitic goats with 1-hour pre-single-dose oral administration of Fibrosin. However, ceftizoxime, a major active metabolite of ceftriaxone, was recovered at a plasma concentration of 23.16 ± 1.48 μg/mL with its parent ceftriaxone 10.12 ± 2.90 μg/mL at 36 hours post doing (Table 3). Ceftizoxime concentration in plasma followed a decreasing and increasing pattern and showed a plasma concentration of 30.23 ± 2.78 μg/mL at 96 hours while ceftriaxone maintained a steady level up to 60 hours and showed its minimum plasma concentration of 5.76 ± 1.50 μg/mL at 84 hours post-dosing. Ceftriaxone concentration in plasma of mastitic goats declined at a faster rate and persisted at a minimum concentration at 0.50 hour post-dosing.

Kinetic Parameters

The kinetic behavior of ceftriaxone followed the "1-compartment open" model in healthy goats, while the kinetic pattern was fitted to "2-compartment open" model in Fibrosin-treated healthy goats and "1-compartment open" model in Fibrosin-treated mastitic goats (Figure 4). The pharmacokinetic parameters are shown in Table 4. The biological half-life ($t_{½}\ \beta$) of ceftriaxone (0.10 ± 0.01 hour) decreased prominently in mastitic goats compared with healthy goats in presence of Fibrosin (0.99 ± 0.06 hour) and healthy goats without Fibrosin treatment (0.21 ± 0.01 hour). The mean Vd_{area} value (0.28 ± 0.01 L/kg) was significantly higher in healthy goats than in mastitic goats (0.20 ± 0.01 L/kg). The Cl_B value was 0.93 ± 0.04 L/kg/h in healthy lactating goats. When elimination of ceftriaxone from the plasma was compared among the 3 different groups, the elimination was faster in mastitic goats with Fibrosin and healthy goats without Fibrosin than in healthy goats treated with Fibrosin.

Table 2. Mean Plasma Concentration (µg/mL) of Ceftriaxone in Healthy Lactating Goats and With 1-Hour Pre-Single-Dose Oral Administration of Fibrosin (1.9 g) in Healthy Lactating and Mastitic Goats After Single-Dose Intravenous Administration at 50 mg/kg Body Weight.

Time (hr)	Healthy lactating	Fibrosin treated healthy lactating	Mastitic
0.08	$135.00^{b} \pm 4.35$	$230.00^{a} \pm 11.54$	$141.00^{b} \pm 14.22$
0.16	$106.91^{b} \pm 7.42$	$180.00^{a} \pm 8.08$	$80.58^{b} \pm 13.03$
0.25	$76.08^{b} \pm 2.70$	$135.33^{a} \pm 4.33$	$47.00^{b} \pm 10.11$
0.33	$54.50^{b} \pm 1.44$	$103.00^{a} \pm 2.51$	$29.86^{b} \pm 7.56$
0.50	$33.75^{NS} \pm 3.03$	$60.26^{NS} \pm 0.37$	$9.46^{NS} \pm 3.26$
0.66	19.16 ± 1.92	40.58 ± 4.75	BDL
1	7.03 ± 1.54	33.66 ± 3.71	BDL
2	BDL	16.60 ± 1.44	BDL
3	BDL	BDL	BDL
4	BDL	BDL	BDL
6	BDL	BDL	BDL
8	BDL	BDL	BDL
12	BDL	BDL	BDL
24	BDL	BDL	BDL

Mean of 6 replicates ± SE.
Means in row bearing at least one common superscript do not differ significantly ($P < 0.05$).
NS = not significant; BDL = below detection limit.

Table 3. Mean Plasma Concentration (µg/mL) of Ceftizoxime (Metabolite of Ceftriaxone) and Ceftriaxone in Healthy Lactating Goats and With 1-Hour Pre-Single-Dose Oral Administration of Fibrosin (1.9 g) in Healthy and Mastitic Goats After Single-Dose Intravenous Administration of Ceftriaxone at 50 mg/kg Body Weight.

		Fibrosin-Treated Healthy Lactating		
Time (hr)	Healthy Lactating	Ceftizoxime	Ceftriaxone	Mastitic
36	BDL	23.16 ± 1.48	10.12 ± 2.90	BDL
48	BDL	18.66 ± 1.24	8.50 ± 2.45	BDL
60	BDL	19.93 ± 1.33	8.87 ± 2.51	BDL
84	BDL	15.36 ± 1.37	5.76 ± 1.50	BDL
96	BDL	30.23 ± 2.78	BDL	BDL
120	BDL	NC	NC	BDL

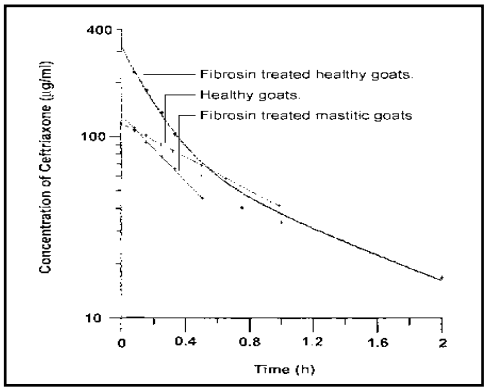
Mean of 6 replicates ± SE.
NC = not collected; BDL = below detection limit.

Milk Concentration

Mean milk concentration of ceftriaxone and its metabolite, ceftizoxime, in healthy lactating goats, Fibrosin-treated healthy lactating goats, and Fibrosin-treated mastitic goats after single-dose intravenous administration of ceftriaxone at 50 mg/kg are presented in Table 5. Milk concentration of ceftizoxime started to increase from 0.08 hour (2.00 ± 0.28 µg/mL) and achieved its peak level at 48 hours (406.61 ± 28.66 µg/mL); this was followed by a slow decline and reached its minimum concentration at 720 hours (18.48 ± 4.38 µg/mL) in healthy lactating goats. Ceftizoxime attained a concentration of 29.00 ± 3.46 µg/mL at 0.08 hour, then started to increase from 3 hours with a peak level of 420.50 ± 31.82 µg/mL at 36 hours; this persisted up to 360 hours post-dosing at higher concentration in milk of goats with a pre-single-dose oral administration of Fibrosin after single-dose intravenous administration of ceftriaxone at 50 mg/kg. Ceftriaxone was also present in milk at 3,

96, and 120 hours post-dosing in these Fibrosin-treated goats. In Fibrosin-treated mastitic goats, ceftriaxone or ceftizoxime concentration could not be detected up to 6 hours post-dosing due to agalactia. Active metabolite ceftizoxime showed a concentration of 69.75 ± 3.17 µg/mL at 8 hours; the concentration increased and decreased at different time intervals. Ceftizoxime concentration was below detectable levels in mastitic goats after 336 hours post-dosing. BTB paper test using milk of mastitic goats showed light green color at 120 hours post-dosing, and all signs and symptoms waned.

Figure 4. Semilogarithmic plot of mean plasma concentration of ceftriaxone against time with computerized best-fit line in healthy lactating goats and with 1-hour pre-single-dose oral administration of Fibrosin (1.9 g) in healthy lactating and mastitic goats after single-dose intravenous administration at 50 mg/kg body weight.



Urinary Excretion

Mean urine concentration of ceftizoxime and ceftriaxone after single-dose intravenous administration of ceftriaxone at 50 mg/kg in healthy lactating, Fibrosin-treated healthy lactating and mastitic goats are reported in Table 6. Urine concentrations of ceftizoxime were 468.61 ± 84.04, 312.85 ± 98.47, and 152.89 ± 41.59 µg/mL at 24, 48, and 72 hours post-dosing in healthy goats after single-dose intravenous administration of ceftriaxone. Urine concentration of ceftriaxone was 531.74 ± 29.68 µg/mL at 24 hours, while ceftizoxime in urine was detected to be 283.63 ± 38.66 and 204.45 ± 40.39 µg/mL at 48 and 72 hours post-dosing, respectively, in Fibrosin-treated healthy goats. Ceftriaxone was present at 24 hours (385.00 ± 51.96 µg/mL), while ceftizoxime excreted at a concentration of 245.00 ± 31.75 and 145.00 ± 23.09 µg/mL through urine of mastitic goats with prior treatment of Fibrosin at 48 and 72 hours, respectively.

Microsomal Study

Cytochrome P450 contents of liver microsome of control (healthy goats without any treatment), goats with single-dose oral administration of Fibrosin (1.9 g), goats given single-dose intravenous administration of ceftriaxone with 1-hour prior single-dose oral administration of Fibrosin (1.9 g) and goats with single-dose intravenous administration of ceftriaxone are shown in Table 7. Cytochrome P450 content (1.06 ± 0.04 n mole/mg of microsomal protein) in goats of control group was inhibited significantly ($P < 0.05$) to (0.685 ± 0.06 n mole/mg of microsomal protein) in goats after 48 hours of single-dose oral administration of Fibrosin. Cytochrome P450 content (1.391 ± 0.12 n mole/mg of microsomal protein) with 1-hour pre-single-dose oral administration of Fibrosin (1.9 g) after 48 hours of single-dose intravenous administration of ceftriaxone at 50 mg/kg was increased significantly from control value. Cytochrome P450 content (1.65 ± 0.16 n mole/mg of microsomal protein) was increased significantly after 48 hours of single-dose intravenous administration of ceftriaxone at 50 mg/kg compared with control and Fibrosin-treated goats.

DISCUSSION

The pharmacokinetic profile of ceftriaxone in healthy, mastitic goats and kinetic interaction with polyherbal drug Fibrosin in healthy and mastitic goats were examined in these experiments. It was observed that ceftriaxone has a limited to moderate distribution, shorter half-life, and major hepatic clearance in both healthy lactating and mastitic goats after single-dose intravenous

Table 4. Mean Kinetic Parameters of Ceftriaxone in Healthy Lactating Goats and With 1-Hour Pre-Single-Dose Oral Administration of Fibrosin (1.9 g) in Healthy Lactating and Mastitic Goats After Single-Dose Intravenous Administration at 50 mg/kg Body Weight.

Kinetic Parameters	Healthy Lactating	Fibrosin-Treated Healthy Lactating	Mastitic
B (µg/mL)	172.25[b] ± 7.66	68.97 ± 9.66	237.23[a] ± 12.26
β (hr^{-1})	3.27[b] ± 0.20	0.70 ± 0.04	6.67[a] ± 0.71
$t_{½}$ Symbol (hr)	0.21[a] ± 0.01	0.99 ± 0.06	0.10[b] ± 0.01
AUC (µg•hr/mL)	52.89 ± 2.82	149.04 ± 7.60	36.69 ± 5.45
Vd_{area} (L/kg)	0.28[a] ± 0.01	0.48 ± 0.04	0.20[b] ± 0.01
Cl_B (L/kg/hr)	0.93[NS] ± 0.04	0.33 ± 0.01	1.39[NS] ± 0.23
Cl_R (L/kg/hr)	—	0.005 ± 0.0005	0.008 ± 0.002
Cl_H (L/kg/hr)	0.93[NS] ± 0.04	0.328 ± 0.01	1.38[NS] ± 0.40
MRT (hr)	0.30[a] ± 0.01	0.88 ± 0.06	0.14[b] ± 0.014
$C°_P$ (µg/mL)	—	344.18 ± 16.34	—
A (µg/mL)	—	275.20 ± 14.12	—
α (hr^{-1})	—	5.39 ± 0.18	—
$t_{1/2}$ Symbol (hr)	—	0.12 ± 0.003	—
Vd_C (L/kg)	—	0.14 ± 0.01	—
Vd_B (L/kg)	—	1.14 ± 0.30	—
Vd_{SS} (L/kg)	—	0.32 ± 0.03	—
K_{12} (hr^{-1})	—	2.14 ± 0.07	—
K_{21} (hr^{-1})	—	1.64 ± 0.16	—
K_{el} (hr^{-1})	—	2.31 ± 0.14	—
f_C	—	0.29 ± 0.02	—
T ~ B	—	2.34 ± 0.23	—

A, B = t_0 plasma drug concentrations intercepts of biphasic intravenous disposition curve; the co-efficient A is the point of intercept of residuals and co-efficient B is based on terminal elimination phase.
Mean of 6 replicates ± SE.
Means in row bearing at least one common superscript do not differ significantly ($P < 0.05$).
NS = not significant.

$C°_P$,theoretical zero time plasma drug concentration; α, rate constant related to slope of absorption curve; β, rate constant related to slope of elimination curve; $t_{½}$ α and $t_{½}$ β, half-lives of the drug in absorption and elimination phases, respectively; Vd_{area}, Vd_B, Vd_{SS} and Vd_C, apparent volume of distribution of the drug on the total area under plasma drug concentration versus time curve, distribution neglecting the absorption phase, distribution at steady state, and distribution in the central compartment, respectively; T ~ B , tissue/blood ratio; f_C , fraction of the amount of drug in the central compartment; Cl_B, total body clearance of drug; K_{el}, first-order rate constant for drug elimination from central compartment; K_{12}, first-order rate constant for transfer of drug from central to peripheral compartment; K_{21}, first-order rate constant for transfer of drug from peripheral to central compartment; AUC, total area under the plasma drug concentration versus time curve from 0 to t α after administration of a single dose; Cl_R, renal clearance of the drug; Cl_H, hepatic clearance of the drug.

administration. Ceftriaxone is widely used in humans due to its prolonged terminal half-life (5.4-8.2 hours). But terminal half-lives of 1.40 and 0.81-1.62 hours have been reported for calves[8] and adult horses,[9] respectively. The lower $t_{½}$ β value of ceftriaxone in goats might be due to species variations. The higher Cl_B or Cl_H value was also responsible for shorter half-life. Hepatic clearance means the drug may either undergo metabolism and/or be excreted through bile. Ceftizoxime is available in milk and urine of ceftriaxone-administered healthy goats, which may suggest metabolism of ceftriaxone to ceftizoxime in liver. The plasma concentration of ceftriaxone increased significantly in Fibrosin-treated healthy lactating goats starting from 0.08 hour to 0.33 hour and persisted with a higher concentration for 2 hours post-dosing; it was present

Table 5. Mean Milk Concentration (µg/mL) of Ceftizoxime (Metabolite of Ceftriaxone) and Ceftriaxone in Healthy Lactating Goats and With 1-Hour Pre-Single-Dose Oral Administration of Fibrosin (1.9 g) in Healthy and Mastitic Goats After Single-Dose Intravenous Administration of Ceftriaxone at 50 mg/kg Body Weight.

	Healthy Lactating	Fibrosin-Treated Healthy Lactating		Mastitic
Time (hr)	Ceftizoxime	Ceftizoxime	Ceftriaxone	Ceftizoxime
0.08	$2.00^{NS} \pm 0.28$	$29.00^{NS} \pm 3.46$	BDL	-
0.16	$3.41^{NS} \pm 0.46$	$3.75^{NS} \pm 0.43$	BDL	-
0.25	$6.16^{NS} \pm 0.60$	$23.00^{NS} \pm 1.73$	BDL	-
0.33	$8.58^{NS} \pm 1.15$	$4.00^{NS} \pm 0.52$	BDL	-
0.50	$24.50^{NS} \pm 2.59$	$27.33^{NS} \pm 1.45$	BDL	-
0.66	$37.33^{NS} \pm 2.90$	$51.00^{NS} \pm 2.30$	BDL	-
1	$40.16^{NS} \pm 2.89$	$5.33^{NS} \pm 1.16$	BDL	-
3	$45.41^{NS} \pm 1.59$	$47.58^{NS} \pm 2.88$	33.33 ± 0.93	-
4	$86.66^{NS} \pm 8.81$	$81.41^{NS} \pm 5.26$	BDL	-
6	$134.79^{NS} \pm 7.57$	$159.00^{NS} \pm 9.23$	BDL	-
8	211.33 ± 16.33	$243.00^{a} \pm 18.47$	BDL	$69.75^{b} \pm 3.17$
12	$258.66^{b} \pm 16.74$	$301.50^{a} \pm 22.66$	BDL	$107.50^{c} \pm 5.51$
24	$230.66^{a} \pm 5.64$	$184.50^{b} \pm 14.86$	BDL	$171.50^{b} \pm 7.90$
36	$386.91^{a} \pm 20.29$	$420.50^{a} \pm 31.82$	BDL	$129.25^{b} \pm 4.67$
48	$406.61^{a} \pm 28.66$	$303.50^{b} \pm 9.74$	BDL	$144.75^{b} \pm 6.87$
72	$383.00^{a} \pm 13.00$	$288.83^{b} \pm 28.77$	BDL	$47.25^{c} \pm 3.0$
96	$332.66^{a} \pm 18.76$	$247.25^{b} \pm 10.32$	35.00 ± 8.08	$24.75^{c} \pm 2.19$
120	$246.66^{a} \pm 11.77$	$122.50^{b} \pm 7.50$	56.00 ± 15.01	$137.75^{b} \pm 7.27$
144	$289.33^{a} \pm 11.11$	$109.16^{c} \pm 13.09$	BDL	$225.50^{b} \pm 11.69$
168	$252.75^{a} \pm 18.00$	$226.00^{a} \pm 10.96$	BDL	$140.00^{b} \pm 8.66$
192	$250.43^{NS} \pm 11.56$	$280.50^{NS} \pm 14.43$	BDL	$230.50^{NS} \pm 14.34$
216	$214.66^{b} \pm 8.66$	$282.00^{a} \pm 11.54$	BDL	$214.00^{b} \pm 11.63$
240	$145.75^{c} \pm 5.93$	$285.00^{a} \pm 20.20$	BDL	$236.75^{b} \pm 17.03$
288	$162.00^{c} \pm 11.54$	$300.00^{a} \pm 20.20$	BDL	$204.00^{b} \pm 12.12$
336	153.00 ± 17.38	320.00 ± 25.20	BDL	225.25 ± 23.16
360	123.66 ± 13.07	364.41 ± 23.09	BDL	BDL
480	61.76 ± 11.65	BDL	BDL	BDL
576	43.00 ± 7.14	NC	BDL	NC
600	30.58 ± 5.70	NC	BDL	NC
720	18.48 ± 4.38	NC	BDL	NC

Mean of 6 replicates ± SE.
Means in row bearing at least one common superscript do not differ significantly ($P < 0.05$).
NC = not collected; NS = not significant; BDL = below detection limit.

for 1 hour in healthy goats without Fibrosin and for 0.50 hour in mastitic goats with 1-hour pre-single-dose oral administration of Fibrosin. The Cl_B, Cl_R, and Cl_H values were recorded to be 0.33 ± 0.01, 0.005 ± 0.0005, and 0.33 ± 0.01 L/kg/h, respectively, in Fibrosin-treated healthy goats, indicating that Fibrosin (polyherbal drug) decreased the body clearance of ceftriaxone. Body clearance includes clearance through different routes and/or metabolism. Ceftriaxone persisted for 0.50 hour post-dosing and its plasma concentration declined at a faster rate in mastitic goats, which might be due to its quick body clearance, even in presence of Fibrosin. Microsomal cytochrome P450 study suggests that ceftriaxone is an inducer of hepatic microsomal enzyme systems, while Fibrosin is the inhibitor of the enzyme; interestingly, combined therapy

Table 6. Mean Urine Concentration (µg/mL) of Ceftizoxime (Metabolite of Ceftriaxone) and Ceftriaxone in Healthy Lactating Goats and With 1-Hour Pre-Single-Dose Oral Administration of Fibrosin (1.9 g) in Healthy Lactating and Mastitic Goats After Single-Dose Intravenous Administration of Ceftriaxone at 50 mg/kg Body Weight.

	Healthy Lactating		Fibrosin-Treated Healthy Lactating		Mastitic	
Time (hr)	Ceftriaxone	Ceftizoxime	Ceftriaxone	Ceftizoxime	Ceftriaxone	Ceftizoxime
0-24	BDL	468.61 ± 84.04	531.74 ± 29.68	BDL	385.00 ± 51.96	BDL
24-48	BDL	312.85 ± 98.47	BDL	283.63 ± 38.66	BDL	245.00 ± 31.75
48-72	BDL	152.89 ± 41.59	BDL	204.45 ± 40.39	BDL	145.00 ± 23.09

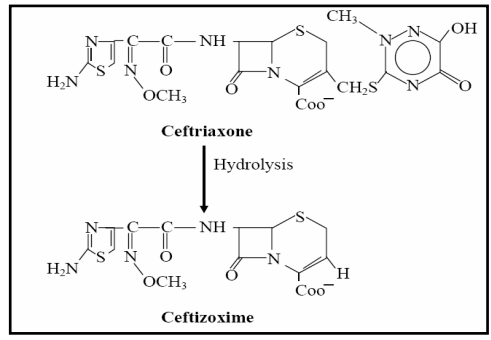
Mean of 6 replicates ± SE.
BDL = below detection limit.

Table 7. Mean Values ± SE of Cytochrome P450 Content (n mole/mg of microsomal protein) of Liver Microsomal Pellet From Healthy Goats of Control and Healthy Goats After Single-Dose Administration of Oral Fibrosin (1.9 g) With Intravenous Ceftriaxone at 50 mg/kg Body Weight (both alone and in combination).

Animal	Control	Oral Fibrosin*	IV Ceftriaxone + Oral Fibrosin*†	IV Ceftriaxone*
Animal1	1.05	0.575	1.630	1.93
Animal2	0.99	0.685	1.195	1.37
Animal3	1.14	0.795	1.350	1.65
Mean ± SE	1.06^{b} ± 0.04	0.685^{c} ± 0.06	1.391^{a} ± 0.12	1.65^{a} ± 0.16

Means bearing at least one common superscript do not differ significantly ($P < 0.05$).
*Goats slaughtered after 48 hours of administration.
†Healthy goats with 1-hour pre-single-dose oral administration of Fibrosin.

induce enzyme activity. Ceftriaxone undergoes hydrolysis through the mixed function oxidase system to produce ceftizoxime and therefore may induce the MFO system. However, because Fibrosin is a polyherbal drug, it is difficult to explain which ingredient may be responsible for inhibition of the MFO system. Combined therapy showed induction of the MFO system, indicating activity of ceftriaxone predominates the activity of Fibrosin. Ceftriaxone undergoes hydrolysis through cleavage of thioether bond present in ceftriaxone.



Proposed metabolic pathway of ceftriaxone

Metabolism of ceftriaxone in these animals warrants further research on metabolism study in other species of animals and human beings.

In conclusion, the results of the present experiment suggest that ceftriaxone along with its major active metabolite ceftizoxime possess excellent antibacterial activity and have high promise for clinical application, particularly in mastitis of livestock animals.

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