



# Pharmacokinetics of ceftiofur in healthy and lipopolysaccharide-induced endotoxemic newborn calves treated with single and combined therapy

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**ABSTRACT.** The aim of this research was to compare plasma pharmacokinetics of ceftiofur sodium (CS) in healthy calves, and in calves with experimentally induced endotoxemia. Six calves received CS (2.2 mg/kg, IM) 2 hr after intravenous administration of 0.9% NaCl (Ceft group). After a washout period, the same 6 calves received CS 2 hr after intravenous injection of lipopolysaccharide (LPS+Ceft group). Another group of 6 calves received a combination of drug therapies that included CS 2 hr after administration of 0.9% NaCl (Comb group). A third group of 6 calves received the same combination therapy regimen 2 hr after intravenous injection of lipopolysaccharide (LPS+Comb group). Plasma concentrations of CS and all desfuoylceftiofur-related metabolites were determined using HPLC, and its pharmacokinetic properties were determined based on a two-compartment model. The peak concentration of CS in the LPS+Comb group occurred the earliest, and the clearance rate of CS was the highest in the Comb and LPS+Comb groups ( $P<0.05$ ). The elimination half-life of CS in the LPS+Ceft group was longer than that in the Ceft and Comb groups ( $P<0.05$ ). The results of this study indicate that combined therapies and endotoxemic status may alter the plasma pharmacokinetics of CS in calves.

**KEY WORDS:** calve, ceftiofur, endotoxemia, pharmacokinetic

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Neonatal calf diarrhea is one of the main causes of mortality in calves. Diarrhea is triggered by lipopolysaccharide (LPS) produced by *Escherichia coli* (*E. coli*). Exposed calves can exhibit sepsis-related symptoms. The primary aims in treating sepsis in calves should be controlling infection, regulating the inflammatory response, replacing fluids, managing circulatory tension and relieving stress [1, 4, 5, 41].

Ceftiofur sodium (CS) is a broad-spectrum, third-generation cephalosporin that was developed for use in animals, and is the most widely recommended parenteral antibiotic for treatment of *E. coli*-induced diarrhea in calves [4, 5, 53]. CS is approved for the treatment of respiratory infections in cattle and pigs [14]. Although previous studies have shown that 2.2 mg/kg of CS is effective in animals, this dosage has not been approved for use in pre-ruminating calves [4]. However, CS can be used for extra-label purposes. Pharmacokinetic studies of CS indicate that it is rapidly absorbed at the site of the injection, and is distributed throughout the body fluid compartments [2, 48]. CS is rapidly metabolized to desfuoylceftiofur (DFC) after administration. DFC, which contains an intact  $\beta$ -lactam ring, is the microbiologically active metabolite of CS [39].

Intravenous administration of LPS can experimentally induce symptoms similar to naturally occurring endotoxemia in animal models of septic shock [6, 54]. The use of intravenous LPS injections in experimental studies is useful for determining dosage regimens of therapeutic drugs for septic shock [12, 13]. Although previous studies have described the pharmacokinetics of various antibiotics for treatment of sepsis and septic shock in humans and in veterinary medicine [13, 38], the pharmacokinetics of CS in subjects with sepsis or septic shock have not been fully characterized.

In critically ill patients, the distribution of hydrophilic antibiotics including  $\beta$ -lactams can broaden, and the pharmacokinetic

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profiles of drugs can become unpredictable, requiring careful consideration of pharmacokinetic parameters when determining the dosage of  $\beta$ -lactam antibiotics [27, 35]. Animal experiments indicate that efficacies of  $\beta$ -lactams for treatment of systemic microbial infections are related to the duration a drug concentration remains above the minimum inhibitory concentration ( $T > MIC$ ), and time-dependent killing is characteristic of  $\beta$ -lactams [29]. Although therapeutic drug level monitoring (TDM) methods are not frequently used for  $\beta$ -lactam treatments, TDM can be used to optimize the dosage of drugs, improve clinical outcomes, and prevent the development of antibiotic resistance when treating critically ill patients for sepsis or other systemic microbial infections requiring a high MIC (minimum inhibition concentration) [37, 44].

The basic aim of the current study was to determine the pharmacokinetic profile of CS with or without combined treatment in endotoxemic calves. We used LPS to experimentally induce endotoxemia in our calf model of septic shock, and monitored the pharmacokinetic parameters of CS and the improvements in clinical and physiological measures that occurred in endotoxemic calves treated with CS alone or CS combined with a vasoactive medication (dopamine), an anti-inflammatory drug (dexamethasone), or intense fluid replacement.

## MATERIALS AND METHODS

### Animals and materials

Eighteen healthy Holstein calves (<6 days old,  $43.54 \pm 5.21$  kg) were used and experimental procedures were approved by the Ethics Committee of the Faculty of Veterinary Medicine at Selcuk University (Konya, Turkey). The animals were divided into two categories, with each category consisting of two groups.

The calves ( $n=6$ ) in the first group of the first category received 2.2 mg/kg (IM) dose of CS (Excenel enj, Pfizer®, Istanbul, Turkey) 2 hr after administration of a 30-min intravenous infusion of 0.9% NaCl (Ceft group). After a 5-day washout period, the calves ( $n=6$ ) in the first group of the first category (cross-over design) were treated with CS (2.2 mg/kg, IM) 2 hr after administration of a 30-min intravenous infusion of 2  $\mu$ g/kg of LPS (*E. coli* 0111:B4, Sigma-Aldrich, Hamburg, Germany) dissolved in 100 ml 0.9% NaCl (LPS+Ceft group).

The calves in the first group ( $n=6$ ) of the second category (parallel design) received combination drug therapy containing [Voluven (20 ml/kg, IV infusion), ringers lactate (20 ml/kg, IV), dexamethasone (10 mg/calf, IV), dopamine (30  $\mu$ g/kg/min, IV), sodium bicarbonate (8.4%, IV), dextrose (5%, IV)] and CS (2.2 mg/kg, IM) 2 hr after administration of a 30-min intravenous infusion of 0.9% NaCl (Comb group). The calves in the second group ( $n=6$ ) of the second category received the same treatment as the Comb group 2 hr after intravenous injection of 2  $\mu$ g/kg of LPS (LPS+Comb group).

### Parameters of endotoxemia model

Body temperature is a critical indicator of septic shock. The body temperature of the calves was measured rectally at 0.5, 1, 1.5 and 2 hr before and 0, 0.5, 1, 2, 4, 6, 8, 10 and 22 hr after administration of CS. Clinical and physiological indicators, including sucking reflex, capillary refill time, defecation interval, respiratory pattern, arterial blood pressure (auricular artery), heart rate (brachial artery), respiratory rate, and body temperature were monitored and recorded at all time points. The physiological parameters were measured using a BM5Vet Monitor (Bionet, Seoul, South Korea). Changes in the parameters observed were scored numerically.

### Sample collection

The body temperature of the calves was measured rectally at 0.5, 1, 1.5 and 2 hr before and 0, 0.5, 1, 2, 4, 6, 8, 10 and 22 hr after the administration of CS. Other clinical and physiological indicators were recorded (Table 1). Physiological parameters were measured using a monitor (BM5Vet Monitor). Changes in parameters were scored numerically. Prior to drug administration, a jugular catheter was inserted into the jugular vein. Blood samples (4 ml) were collected from the jugular vein in lithium heparin vacuum tubes at 5, 10, 20, 30, 45, 60 and 90 min before and 2, 3, 4, 6, 8, 10, 22, 34 and 46 hr after administration of CS. Blood samples were centrifuged at 3,000 g for 10 min to obtain plasma, and plasma samples were stored at  $-70^{\circ}\text{C}$ .

**Table 1.** HPLC-UV double gradient elution scheme

Time (min)	Flow rate (ml/min)	A (%)	B (%)
0.01	0.3	10	90
4	0.3	30	70
6	0.3	25	75
8	0.3	25	75
11	0.3	18	82
16	0.3	10	90
20	0.3	10	90

A, acetonitrile; B, 0.1% formic acid in water.

### CS and desfuroylceftiofur-related metabolite analysis

CS and all desfuroylceftiofur-related metabolites (DFC) in plasma samples were quantitated using high-pressure liquid chromatography-ultraviolet (HPLC-UV) spectrophotometry (Shimadzu, Tokyo, Japan). The HPLC system consisted of a low-pressure-gradient flow control valve (FCV-10AL), pump (LC-10AD), system control module (CBM 20A), degasser (DGU-14A), auto sampler (SIL-10AD), column oven (CTO-10A), and UV-VIS detector (SPD-10A). Separation was achieved using a PLRP-S column (150 × 2.1 mm; internal diameter, 3 μm; Agilent technologies). The mobile phase, which consisted of acetonitrile (Merck, Darmstadt, Germany) (A) in water (B) with 0.1% formic acid (Merck), was filtered through a 0.4-μm nylon filter (0.45 μm, Millipore, Bedford, MA, U.S.A.), and sonicated (Elma, Singen, Germany) for 30 min. A gradient elution was performed using a flow rate of 0.3 ml/min and an injection volume of 20 μl (Table 1). The column oven was set at 40°C, and the eluate was scanned at 266 nm for peak detection. The retention time was approximately 11 min, and the total analysis time was 20 min.

CS and DFC were quantitated according to a previously published method [50]. After administration of CS, it is rapidly converted to the biologically active metabolite, DFC. Therefore, plasma DFC concentrations were determined for the pharmacokinetics of CS. In this method, CS is extracted from plasma samples using a derivitization method that converts CS and all metabolites to DFC. In brief, about 200 μl of each plasma sample was brought to room temperature and transferred to 2 ml microcentrifuge tubes. Two-hundred microliters of methanol was added, and samples were vortexed for 30 sec. After centrifugation of the sample at 13,000 g for 10 min at 22°C, clear supernatant was transferred to 2 ml microcentrifuge tubes, 100 μl of 10% dithioerythritol in borate buffer was added to each tube, and each tube was placed in a water bath at 50°C for 15 min. Tubes were moved from the water bath and allowed to reach room temperature. Next, 100 μl of 23.3% iodoacetamide in phosphate buffer was added to each tube, tubes were wrapped in aluminum foil and shaken at 350 rpm for 45 min at room temperature. Twenty-five microliters of formic acid was added to each sample. Following derivitization, samples were vortexed for 30 sec after stirring at 22°C, and were centrifuged for 10 min at 13,000 rpm. An aliquot of the resulting supernatant (20 μl) was injected into the HPLC-UV system. All chemicals and solvents used in our experiments were HPLC-grade.

The CS standards were prepared from a 1 mg/ml stock solution of CS by diluting 0.05 and 20 μg/ml solutions of CS with 190 and 10 μl of water, respectively. The assay was linear over the concentration range of 0.05 to 20 μg/ml. The limits of quantification (LOQ) and detection (LOD) of the method were found to be 0.1 and 0.05 μg/ml in plasma, respectively. LOD was defined as the lowest amount of analyte detectable in the plasma at which the signal/noise ratio in a sample is at least 3. LOQ is defined as the lowest computable concentration of the analyte in the plasma with a signal/noise ratio of at least 10. Intraday percent coefficients of variation (CV) (n=18) were as follows: 0.5 μg/ml, 3.63%; 2 μg/ml, 2.81%; 10 μg/ml, 10.05%. Interday per cent CV for CS in plasma (n=54) were as follows: 0.5 μg/ml, 4.36%; 2 μg/ml, 1.70%; 10 μg/ml, 5.93%. The coefficient of variation was calculated as CV (%)=(standard deviation/mean) × 100.

### Data processing

The plasma concentration-time curve was constructed for each calf using the software program (Phoenix WinNonLin 6.3, Pharsight, St. Louis, MO, U.S.A.). Individual plasma concentration-time curves and Akaike Information Criteria (AIC) were examined for evaluation of the pharmacokinetics of CS and DCF, as previously described [52]. The pharmacokinetic variables were analyzed based on a two-compartment model. The peak plasma concentration ( $C_{max}$ ), and time elapsed to reach peak plasma concentration ( $T_{max}$ ) were determined based on the plasma concentration-time curve for each calf. Other parameters were calculated using the pharmacokinetic software program (Phoenix WinNonLin 6.3, Pharsight).

### Statistical analysis

The statistical differences among parameters were determined using SPSS, version 19.0, software. Differences in body temperature were assessed using analysis of variance (ANOVA) and Tukey tests. The mean harmonic value was calculated for the time parameters ( $t_{1/2\alpha}$ ,  $t_{1/2\alpha}$  and  $t_{1/2\beta}$ ), which compared using the Mann-Whitney *U* test. Differences among the other parameters were evaluated using ANOVA and Tukey tests (SPSS 22.0).  $P < 0.05$  was accepted to represent statistical significance.

## RESULTS

### Parameters of endotoxemia model

The sucking reflex, respiratory activity and general health indicators of the animals changed significantly following treatment with LPS, with signs of septic shock detectable within 1 hr following LPS-induction. However, the calves had recovered from these pathophysiological changes by the last time point post LPS-induction. Following LPS treatment, capillary refill time increased, defecation interval decreased, and each animal's overall condition markedly deteriorated. Irregular decreases in arterial blood pressure and erratic changes in pulse were observed in the LPS+Ceft group. Irregular increases in body temperature and respiration rate were observed in calves treated with LPS (Table 2).

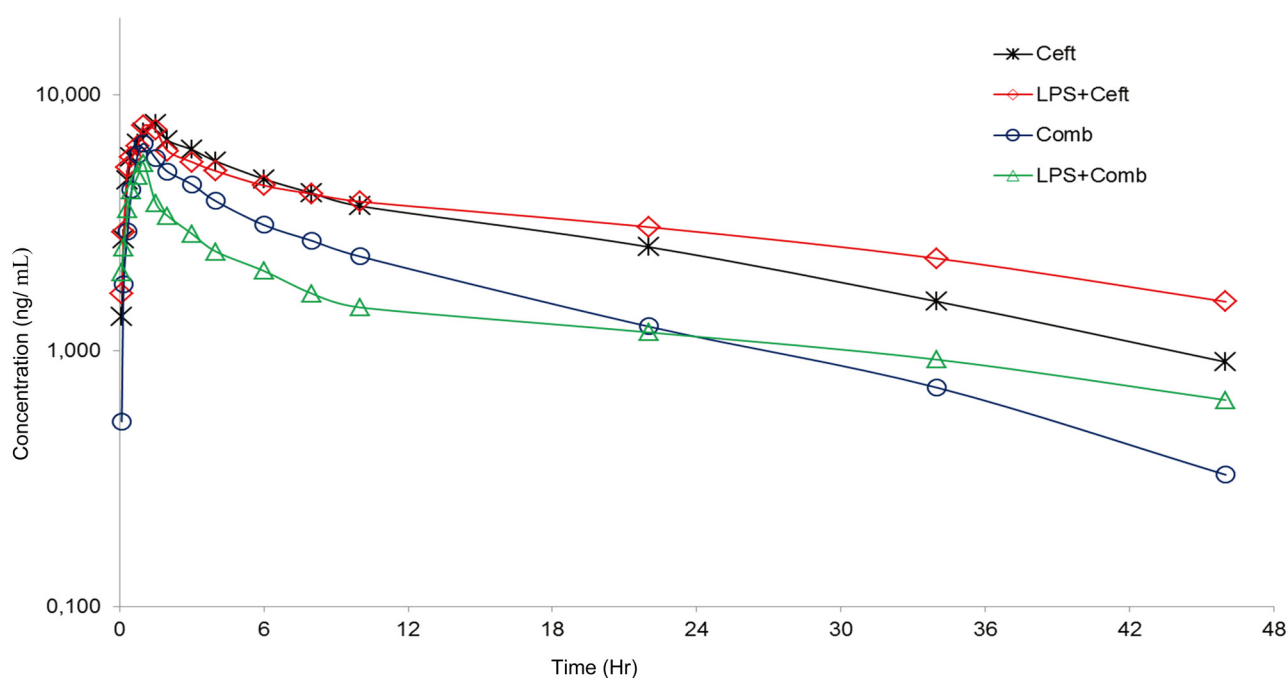
### Pharmacokinetics

Figure 1 illustrates the plasma concentration-time curves of each group. Pharmacokinetic properties were calculated based on a two-compartment model. The pharmacokinetic parameters of CS and DFC are given in Table 3. A lower  $C_{max}$  was observed ( $P < 0.05$ ) in the LPS+Comb group than in the Ceft and LPS+Ceft groups;  $K_a$  and  $t_{1/2\alpha}$  of the Comb group were statistically different ( $P < 0.05$ ) from those of the LPS treated groups and all other groups, respectively. The  $t_{1/2\beta}$  of the LPS+Ceft group was longer

**Table 2.** General clinical data for healthy calves and experimentally induced endotoxemic calves that received ceftiofur sodium treatment or combination therapy

	Variables	Ceft	LPS+Ceft	Comb	LPS+Comb
Clinical scoring <sup>a)</sup>	Sucking reflex	Normal	No	Normal	Improvement
	Capillary refill	Normal	Cyanotic	Normal	Altered light
	Defecation interval	Normal	Short	Normal	Normal
	Respiration type	Normal	Changed	Normal	Altered light
	General health condition	Normal	Changed	Normal	Altered light
Monitoring <sup>a)</sup>	Arterial blood	Normal	Irregular reduction	Normal	Normal
	Pressures pulses	Normal	Irregular promotion	Normal	Irregular promotion
	Body temperature	Normal	Irregular promotion	Normal	Irregular promotion
	Respiratory rate	Normal	Irregular promotion	Normal	Irregular promotion

a) General group assessment was performed based on the results of the statistical analysis.



**Fig. 1.** Semilogarithmic plots of mean plasma concentrations of ceftiofur and desfuroylceftiofur versus time after intramuscular administration of ceftiofur sodium (2.2 mg/kg) in healthy calves (ceftiofur and combined treatment) and experimentally induced endotoxemic calves (Lipopolysaccharide+ceftiofur and Lipopolysaccharide+combined treatment).

( $P < 0.05$ ) than that of the Ceft and Comb groups, while the CL/F values of the Comb and LPS+Comb groups were larger ( $P < 0.05$ ).

### Pharmacodynamics

Bacterial isolates having an MIC value  $< 2.0 \mu\text{g/ml}$  are considered sensitive to CS according to current CLSI guidelines (National Committee for Clinical Laboratory Standards, 1999).

## DISCUSSION

### Parameters of the endotoxemia model

Neonatal calf diarrhea is an important health problem in calves. In the current research, the calves in LPS-treated groups showed some of the clinical and physiological signs of endotoxemia (Table 1). Previous studies have shown that intravenous injection of *E. coli* toxin in calves leads to changes in sucking reflex, capillary refilling time, body temperature, respiration rate, and heart rate [23, 43]. In our current study, a combination therapy initiated 2 hr after LPS injection prevented frequent defecation, and decreased the adverse effects of endotoxemia on capillary refilling time, sucking reflex and general health indicators. Our results are consistent with those obtained in other studies of the treatment of sepsis in calves using various therapeutic agents [4, 28]. Similar results have been reported in previous studies [1, 6]. These results indicate that LPS treatment can mimic endotoxemia.

**Table 3.** Pharmacokinetic parameters of desfuroylceftiofur after ceftiofur sodium injection single dose (2.2 mg/kg, IM) in healthy calves and experimentally induced endotoxemic calves

Parameters	Ceft	LPS+Ceft	Comb	LPS+Comb
$K_a$ (1/hr)	1.86 ± 0.45 <sup>ab</sup>	2.18 ± 0.66 <sup>a</sup>	1.21 ± 0.25 <sup>b</sup>	2.52 ± 0.78 <sup>a</sup>
$t_{1/2a}$ (hr) (HM)	0.37 ± 0.09 <sup>b</sup>	0.32 ± 0.17 <sup>b</sup>	0.57 ± 0.12 <sup>a</sup>	0.27 ± 0.10 <sup>b</sup>
$T_{max}$ (hr)	1.5 <sup>a</sup>	1.5 <sup>a</sup>	1 <sup>b</sup>	1 <sup>b</sup>
(Interval)	(1.5)	(1–1.5)	(1–1.5)	(0.75–1)
$C_{max}$ (μg/ml)	7.73 ± 0.81 <sup>a</sup>	7.86 ± 0.98 <sup>a</sup>	6.54 ± 0.85 <sup>ab</sup>	5.74 ± 1.47 <sup>b</sup>
AUC <sub>0-∞</sub> (hr*μg/ml)	153 ± 26 <sup>b</sup>	223 ± 41 <sup>a</sup>	100 ± 17 <sup>c</sup>	86 ± 17 <sup>c</sup>
$\alpha$ (1/hr)	0.59 ± 0.32	0.70 ± 0.48	0.98 ± 0.21	1.19 ± 0.59
$t_{1/2\alpha}$ (hr) (HM)	1.17 ± 1.26	0.99 ± 0.69	0.71 ± 0.16	0.58 ± 0.48
V1/F (ml/kg)	212 ± 22 <sup>ab</sup>	220 ± 56 <sup>ab</sup>	189 ± 36 <sup>b</sup>	281 ± 75 <sup>a</sup>
V2/F (ml/kg)	182 ± 64 <sup>b</sup>	254 ± 168 <sup>b</sup>	342 ± 97 <sup>b</sup>	568 ± 126 <sup>a</sup>
$K_{10}$ (1/hr)	0.07 ± 0.01 <sup>bc</sup>	0.05 ± 0.02 <sup>c</sup>	0.12 ± 0.03 <sup>a</sup>	0.10 ± 0.03 <sup>ab</sup>
$K_{12}$ (1/hr)	0.26 ± 0.17 <sup>b</sup>	0.38 ± 0.31 <sup>ab</sup>	0.56 ± 0.11 <sup>ab</sup>	0.77 ± 0.41 <sup>a</sup>
$K_{21}$ (1/hr)	0.30 ± 0.16	0.30 ± 0.18	0.34 ± 0.14	0.36 ± 0.18
$\beta$ (1/hr)	0.04 ± 0.01 <sup>a</sup>	0.02 ± 0.00 <sup>b</sup>	0.04 ± 0.01 <sup>a</sup>	0.03 ± 0.01 <sup>ab</sup>
$t_{1/2\beta}$ (hr) (HM)	19.90 ± 3.46 <sup>b</sup>	32.56 ± 6.11 <sup>a</sup>	17.90 ± 2.54 <sup>b</sup>	23.39 ± 5.88 <sup>ab</sup>
CL/F (ml/hr/kg)	14.70 ± 2.57 <sup>b</sup>	10.16 ± 2.24 <sup>b</sup>	22.52 ± 4.19 <sup>a</sup>	26.68 ± 7.81 <sup>a</sup>
$K_{12}/K_{21}$	0.86 ± 0.31 <sup>b</sup>	1.25 ± 0.92 <sup>ab</sup>	1.84 ± 0.55 <sup>ab</sup>	2.12 ± 0.69 <sup>a</sup>
V1/F+V2/F (ml/kg)	394 ± 67 <sup>b</sup>	474 ± 162 <sup>b</sup>	532 ± 112 <sup>b</sup>	850 ± 169 <sup>a</sup>

a, b, c) Varied characters in the same row are statistically significantly different ( $P < 0.05$ ).  $K_a$ : absorption rate constant,  $t_{1/2a}$ : absorption half-life,  $T_{max}$ : time to reach the maximum concentration,  $C_{max}$ : peak plasma concentration, AUC: area under curve,  $\alpha$ : distribution rate constant,  $t_{1/2\alpha}$ : distribution half-life, V1/F: central compartment volume of distribution, V2/F: peripheral compartment volume of distribution,  $K_{10}$ : elimination rate constant of central compartment,  $K_{12}$ : transfer rate constant from central compartment to peripheral compartment,  $K_{21}$ : transfer rate constant from peripheral compartment to central compartment,  $\beta$ : elimination rate constant, CL/F: total clearance,  $t_{1/2\beta}$ : elimination half-life, HM: harmonic means, Ceft: ceftiofur, LPS+Ceft: lipopolysaccharide + ceftiofur, Comb: combined treatments, LPS+Comb: lipopolysaccharide + combined treatments.

### Pharmacokinetics/pharmacodynamics

*In vivo*, the systemic half-life of ceftiofur is shorter than 10 min due to rapid degradation of thioester bonds and rapid conversion into DFC, which is the active metabolite in the body. The beta-lactam ring belonging to ceftiofur remains unchanged in DFC [20, 39]. Conversion of ceftiofur to DFC is followed by derivatization, which stabilizes DFC. Therefore, total antibacterial activity related to ceftiofur and its metabolites correlates with DFC concentration in plasma samples.

The definition of a drug-drug interaction (DDI), affecting pharmacokinetics and/or pharmacodynamics, is a change in the effects of one drug induced by the presence of another drug [30]. Drug-drug interactions are more likely to be seen in patients with reduced absorption, disrupted metabolism, renal insufficiency and polymedication [31]. DDIs, which occur in pharmacokinetic stages such as absorption, distribution, metabolism and excretion, are caused by significant changes in pharmacokinetics of drugs [17, 19]. The liver is the most effective organ at biotransformation of drugs. Drugs metabolized via the liver can be affected by physiological processes such as liver blood flow rate, enzyme activity and binding to proteins [26]. Co-administered drugs have different effects on the activity of drug metabolizing enzymes. In addition, endotoxins produced in response to bacterial inflammatory mediators have different effects on these enzymes [9]. The inhibition or induction of enzymes responsible for chemical modification of drugs indirectly changes the pharmacokinetic profile of the drug [17]. In this study, changes in the pharmacokinetic processes of CS by LPS-induced endotoxemia and combination treatment may be related to changes in metabolite composition of CS. However, determining the DDI in patients receiving combination drug therapy for LPS may be a very complex task.

In this study, we investigated the pharmacokinetics of intramuscularly administered CS in healthy calves and calves with experimentally induced septic shock (Table 3). The results of our analysis of the pharmacokinetic parameters of CS, as well as those of previous studies performed in healthy calves [2] were consistent with a two-compartment open model (Fig. 1). Pharmacokinetic properties of CS in healthy calves are consistent with Brown *et al.* [2]. Although the pharmacokinetics of CS have not been described in endotoxemic calves treated with CS alone or in combination therapy, another cephalosporin has been examined in calves [21]. The calves in the LPS+Comb group have a large CL/F and increased V1/F+V2/F compared to those in the LPS+Ceft group ( $P < 0.05$ , Table 3). These results may indicate that combined therapy alters the pharmacokinetics of CS in endotoxemic calves.

*In vivo*, ceftiofur is rapidly metabolized to DFC, which is the microbiologically active metabolite of CS [20, 39]. In several other studies [3, 10, 18, 51] the dose values of ceftiofur used to calculate pharmacokinetic parameters did not use the total amount of DFC. In this method, conversion of ceftiofur to DFC is followed by derivatization to stabilize DFC. Therefore, total antibacterial activity related to ceftiofur and its metabolites is presented with DFC concentration in plasma samples. In this method, metabolites, including desfuroylceftiofur–protein conjugates, are converted into desfuroylceftiofuracetamide (DCA), which is then quantified

by HPLC. Therefore, in this study, pharmacokinetic parameters were determined by DFC concentrations in plasma at different time points.

The  $K_a$  in the Comb group was significantly lower than that of the LPS+Ceft and LPS+Comb groups ( $P<0.05$ ; Table 3). The  $t_{1/2a}$  in the Comb group was significantly longer than that of the other groups ( $P<0.05$ ; Table 3). The  $T_{max}$  values in the Comb and LPS+Comb groups were significantly shorter ( $P<0.05$ ), and the  $C_{max}$  was significantly lower in the LPS+Comb group, compared with that in the other groups ( $P<0.05$ ). In a previous study, the  $t_{1/2a}$  of intramuscularly administered oxytetracycline was reduced in calves with experimentally induced endotoxemia [24], and the  $t_{1/2a}$  of intramuscularly administered levofloxacin was prolonged, compared with the  $t_{1/2a}$  for these drugs in healthy calves [25]. Absorption of CS in the Comb group was significantly slowed, because  $K_a$  was low and  $t_{1/2a}$  was long. Our current findings suggest that, if CS is to exert its effect rapidly, it should not be administered as a component of combined therapy via a route that involves absorption processes. However, differences in CS absorption processes including  $C_{max}$  between normal and other groups would not be of particular note.

The  $V1/F+V2/F$  in the LPS+Comb was significantly larger than that of the Ceft group ( $P<0.05$ ; Table 3). In addition, the ratio of the rate constants for transfer from the central compartment to peripheral compartments over that for transfer from peripheral compartments to the central compartment ( $k_{12}/k_{21}$ ) in the LPS+Comb group ( $2.12 \pm 0.69$ ) was higher than that of the Ceft group ( $0.86 \pm 0.31$ ,  $P<0.05$ ; Table 3). Recent studies have reported that the distribution volume and the  $C_{max}$  of meropenem, ceftazidime, cefepime, piperacillin-tazobactam, piperacillin, amikacin, and vancomycin increase and decline respectively in sepsis/septic shock patients receiving fluid therapy and catecholamine in intensive care units (ICU) [7, 42, 46]. Abundant intravenous fluid therapies that increase extracellular compartment fluid may cause a significant increase in distribution [40]. Elevated distribution volumes of CS and cefepime have been shown in experimentally infected pigs and calves [32, 47]. Combination therapies including vasopressor drugs and abundant intravenous fluid therapies may lead to an increase in distribution of CS by causing rapid transfer of the drug into the tissues and prolonging elimination in endotoxemic calves.

In our current study, the Ceft group was used as a control group. We observed that the  $t_{1/2\beta}$  in the LPS+Ceft group was significantly longer ( $P<0.05$ ) than that in the Ceft group, but was similar to that in the Comb and LPS+Comb groups ( $P<0.05$ , Table 3). In addition, the  $t_{1/2\beta}$  in the LPS+Ceft group was significantly longer than in the other groups ( $P>0.05$ ), and the  $CL/F$  in the LPS+Ceft group was meaningfully elevated, compared with that in the other groups ( $P>0.05$ ). We also found that the  $CL/F$  in the Comb and LPS+Comb groups was significantly higher than that of the LPS+Ceft and Ceft groups ( $P<0.05$ ; Table 3). Recent studies have shown that plasma half-life and clearance of CS in experimentally infected pigs were longer than in healthy pigs [47]. A study of septic patients treated with ceftriaxone showed that the clearance time of drug increased when used in combination with hemodynamically active drugs [22]. Also, the clearances of ceftazidime and cefepime were higher in patients with septic shock, compared with those in healthy subjects [34]. Blood pressure is increased by vasopressor drug application and fluid therapy, which are provided to achieve a perfusion pressure and maintain adequate blood flow [8]. Intravenously administered dopamine elevates mean arterial blood pressure of calves by increasing vascular resistance [16]. The use of high-dose dexamethasone has been shown to increase survival rate [15] by reducing oxidative stress and organ damage [54]. These findings indicate that CS was excreted faster in the combination therapy group, whereas in the LPS+Ceft group, the rate of excretion slowed down due to circulatory and renal failures that develop during septic shock and extend the half-life of drugs, particularly those that are excreted by the kidneys. However, the current study indicates that combination drug therapy can help regulate the half-life of CS in endotoxemic calves.

The activity of beta-lactams including CS is characterized by time-dependent killing, and the MIC is an indicator of the potency of an antimicrobial drug against a pathogen [11]. The most useful and predictable parameter for therapeutic efficacy of beta-lactams is the percentage of time its concentration remains above MIC (%T>MIC) [27, 38, 49]. It has been reported that %T >4xMIC for maximal bactericidal activity in beta-lactams is shown to be maintained longer (>100%) in critically ill patients and patients with compromised immune responses [33, 45]. Bacterial isolates having an MIC value  $\leq 2.0 \mu\text{g/ml}$  are considered sensitive to CS according to current CLSI guidelines (National Committee for Clinical Laboratory Standards, 1999). Based on previous studies, a dose regimen of 6.6 mg/kg of CS administered subcutaneously is effective for treatment of severe septic disease in neonatal calves and foals according to the MIC  $\leq 2.0 \mu\text{g/ml}$  for >90% of the 12-hr study duration [18, 51]. Therefore, the choice of 2.0  $\mu\text{g/ml}$  as the most effective concentration of CS for endotoxemic shock resulting from *E. coli* appears to be suitable according to current data and CLSI. This pharmacodynamics target was achieved for the major pathogens identified in endotoxemic shock, with effective concentrations of CS observed in plasma for 22, 34, 10 and 6 hr intervals, respectively, in the Ceft, LPS+Ceft, Comb and LPS+Comb groups. In Ceft and LPS+Ceft groups, IM administration of CS at 2.2 mg/kg reached plasma concentrations  $\geq 2.0 \mu\text{g/ml}$  for >80% of the 46 hr study duration, while T >MIC above 80% is not achieved with the 2.2 mg/kg body weight of CS applied with combined therapy for bacteria with MIC value  $\leq 2.0 \mu\text{g/ml}$  at 46 hr interval (Fig. 1). However, the %T >MIC is shown to be maintained for 100% of the dosing interval during treatment of patients in critical condition with septic shock [33, 36]. In this instance, following administration at a dose of 2.2 mg/kg, CS does not maintain a T >MIC above 100% for bacteria with MIC value  $\leq 2.0 \mu\text{g/ml}$  for a 46 hr interval in Comb and LPS+Comb groups. However, the same dose (2.2 mg/kg, IM) maintains this target dosage for 34 hr and 6 hr intervals, respectively, in LPS+Ceft and LPS+Comb groups (Fig. 1). The T > 4xMIC of CS should be sustained across dosing intervals for effective treatment of sepsis in newborn calves. Therefore, the total plasma concentration of CS in this study did not achieve %T >4xMIC for bacteria with MIC value  $\leq 2.0 \mu\text{g/ml}$ .

In conclusion, the present study indicates that combined therapy may alter the plasma pharmacokinetic of CS in healthy and endotoxemic calves and an experimentally induced model of shock can generate the same types of pathological changes observed during actual disease. The pharmacokinetic data of CS obtained from this study are applicable to the treatment of newborn calves with sepsis. The results of this study suggest that a 2.2 mg/kg (IM) dose of CS every 6 hr with combined therapy may provide

plasma concentrations above the therapeutic target of 2.0 µg/ml in endotoxemic new born calves. However, 2.2 mg/kg (IM) dose of CS could not provide plasma concentrations above the therapeutic target of 2.0 µg/ml in critical patients with septic shock. The dose and dose interval of CS may be increased and reduced in newborn calves with combined therapy and endotoxemic status, respectively. The investigation of pharmacokinetics for farm animals in disease state and undergoing a course of medication may provides a rationale for practical adjustment of established dose regimes.

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