Ceftiofur Derivatives in Serum, Uterine Tissues, Cotyledons, and Lochia after Fetal Membrane Retention

M. Drillich,^{*1} S. Arlt,^{*} S. Kersting,^{*} A. A. Bergwerff,[†] P. Scherpenisse,[†] and W. Heuwieser^{*}

^{*}Clinic for Reproduction, Faculty of Veterinary Medicine, Free University of Berlin, Königs Weg 65, D-14163 Berlin, Germany

[†]Division of Public Health and Food Safety, Institute for Risk Assessment Sciences, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80.175, NL-3508 TD, Utrecht, the Netherlands

ABSTRACT

The objective of the study was to determine concentrations of ceftiofur derivatives after subcutaneous application of ceftiofur hydrochloride in cows with retained fetal membranes. Concentrations of ceftiofur derivatives detected as desfuroylceftiofuracetamide were determined in blood serum, endometrium, caruncles, cotyledons, and lochia during 72 h. After induction of parturition, 2 primiparous and 4 multiparous cows having retained fetal membranes for at least 12 h were studied. All cows received 3 consecutive injections (C1 to C3; 24 h apart) of 1-mg ceftiofur equivalents per kilogram of body weight as ceftiofur hydrochloride sterile suspension. Samples of blood, endometrium, caruncles, cotyledons, and lochia were collected immediately before each injection (0 h) and again at 4, 12, and 24 h after C1, C2, and C3. Blood samples were collected from coccygeal vessels. Caruncles were removed from the uterine lumen by manual extirpation and separated from cotyledons. Endometrial tissue (0.5 g) was collected by using Kenny's biopsy apparatus. For all samples, concentrations of potentially active ceftiofur derivatives were quantified using an HPLC assay. Within 2 h (serum), 4 h (endometrium), and 12 h (caruncles, cotyledons, lochia) after C1 and during the entire study period, mean concentration of ceftiofur derivatives exceeded the reported minimum drug concentrations required to inhibit the growth of 90% of isolates for relevant bacteria such as *Escherichia coli*, *Fusobacterium necrophorum*, and *Arcanobacterium pyogenes*. Only in single samples did concentrations decrease temporarily below the reported minimum drug concentrations required to inhibit the growth of 90% of isolates.

Key words: retained fetal membrane, ceftiofur

INTRODUCTION

Cephalosporins are an important class of antimicrobial agents in use today for humans and animals (Hor-

nish and Kotarski, 2002). The third-generation cephalosporin ceftiofur has been developed exclusively for veterinary use. Ceftiofur is approved for treatment of respiratory diseases in ruminants, swine, and horses and for foot rot and acute postpartum metritis in cattle. In Europe, ceftiofur is approved for treatment of acute postpartum metritis during the first 10 d postpartum at a dosage of 1 mg/kg of BW, whereas in the United States it is approved at a dosage of 2.2 mg/kg of BW. The recommended treatment period is 5 consecutive days. Clinical efficacy of a systemic antibiotic treatment with ceftiofur in cows with acute metritis (Smith et al., 1998; Drillich et al., 2001; Zhou et al., 2001; Chenault et al., 2004) and retained fetal membranes (Risco and Hernandez, 2003; Drillich et al., 2003, 2006a,b) has been demonstrated. Common bacteria involved in postpartum uterine diseases are *Escherichia coli*, *Arcanobacterium pyogenes*, and anaerobic species (e.g., *Fusobacterium necrophorum* and *Prevotella* species; Sheldon and Dobson, 2004). The clinical findings in cows with retained fetal membranes (**RFM**) are similar to findings in cows with acute metritis (i.e., increased rectal temperature and fetid vaginal discharge). Therefore, it can be assumed that in most instances RFM is associated with acute metritis.

Although early postpartum use of ceftiofur has been described in several studies, there is only limited information about the pharmacokinetics of ceftiofur in bovine uterine tissue. Pharmacokinetics and bioequivalence of ceftiofur in plasma have been compared for intramuscular and subcutaneous administration (Brown et al., 2000). Previous authors suggested similar therapeutic efficacy for the 2 routes of administration. The in vitro metabolism from ceftiofur to desfuroylceftiofur in bovine kidney, lung, liver, and muscle has been described (Olson et al., 1998). Okker et al. (2002) presented the pharmacokinetics of ceftiofur in plasma, lochia, and uterine tissues of 4 healthy cows. Data were collected during 24 h after a single subcutaneous administration of 1 mg/kg of ceftiofur. Concentrations of ceftiofur derivatives in uterine tissues exceeded the reported minimum drug concentrations required to inhibit the growth of 90% of isolates (**MIC**₉₀) of 0.5 µg/

Received February 2, 2006.

Accepted April 18, 2006.

¹Corresponding author: author@bestandsbetreuung.de

mL for *E. coli* (Cervantes et al., 1993; Salmon et al., 1996; Sheldon et al., 2004) and of 0.125 µg/mL for *A. pyogenes*, *F. necrophorum*, and *Prevotella melaninogenica* (Sheldon et al., 2004). Okker et al. (2002) hypothesized that concentrations of ceftiofur would be greater in cows having acute metritis than in the healthy cows included in their study. This hypothesis is based on findings that ceftiofur accumulates in bacterially induced inflammatory sites (Clarke et al., 1996).

The objective of the present study was to determine concentrations of ceftiofur derivatives in serum, endometrium, caruncles, cotyledons, and lochia of cows having RFM during 72 h after treatment.

MATERIALS AND METHODS

Experimental Design

The study was conducted between February and September 2004 at the Clinic for Reproduction, Faculty of Veterinary Medicine, Free University of Berlin, Germany. Cows were housed at the clinic in a tie-stall barn with straw bedding. Parturition was induced between 270 and 280 d of gestation by using dexamethasone (4 mg/100 kg of estimated BW, Dexasel, Selectavet, Otto Fischer, Weyarn-Holzolling, Germany). A total of 6 Holstein-Friesian cows (2 primiparous and 4 multiparous) having RFM for more than 12 h were studied. Average time from parturition to enrollment was 24.8 h (minimum = 12 h; maximum = 43 h).

During 14 d before calving and until the end of the study period, none of the cows included in the study received antimicrobial or antiinflammatory drugs other than ceftiofur hydrochloride. Study period was defined as the time from between collection of the first sample until collection of the last sample, 24 h after the third of 3 daily injections of ceftiofur.

Ceftiofur (Excenel RTU, Pfizer Animal Health, Karlsruhe, Germany) was administered subcutaneously in the neck region at a dosage of 1 mg of ceftiofur equivalents/kg of estimated BW as ceftiofur hydrochloride sterile suspension. A new vial was opened for each cow entering the study. Cows received ceftiofur on 3 successive days between 0800 and 0900 h (C1, C2, and C3, respectively). Rectal body temperature was measured in all cows immediately before collection of samples.

Baseline samples (0 h) were collected immediately before each injection of ceftiofur hydrochloride (C1). Additional samples were collected 2, 4, 12, and 24 h after C1, C2, and C3. For each cow and at each time point, first blood and then lochia, placentomes, and endometrial tissue were collected.

Methods of Sampling

Blood samples were collected from coccygeal vessels in sterile vacuum tubes (Venoject, Terumo Europe N.V., Leuven, Belgium). Thereafter, samples were stored for a maximum of 4 h at 4°C and centrifuged at $3,500 \times g$ for 10 min at 4°C. Serum was stored at -20°C in 2 aliquots of 2 to 3 mL each until analysis. Before collection of samples from the uterus, the perineum and vulva were cleaned with a paper towel. Thereafter, one sterile-gloved hand was introduced through the vagina into the uterine lumen and collected at least 15 mL of lochia. Samples were collected into 20-mL plastic tubes. After sampling of lochia, one placentome was removed manually from the uterus. Once excised, cotyledons were carefully separated from the caruncles. Cotyledons and caruncles were cleaned and dried with a towel and stored in a plastic tube. Finally, endometrial tissue was sampled by Kenny's biopsy apparatus, which was introduced and guided by hand into the uterus. For each sample, approximately 0.5 g of endometrial tissue was collected, dried, and stored in plastic tubes. All samples of uterine tissues, cotyledons, caruncles, and lochia were stored immediately after sampling at 4°C for a maximum of 4 h before they were frozen and stored at -20°C until analysis.

From one additional cow having RFM, but not treated with ceftiofur hydrochloride nor any other antibiotic, 40 mL of serum; 40 mL of lochia, caruncular, and cotyledonal tissue; and 5 g of endometrium were collected 24 h after calving as blank material for HPLC analysis.

Analytical Methods

Concentrations of ceftiofur residues were determined in caruncles, endometrium, and serum (Okker et al., 2002). In this method, residues of ceftiofur, including metabolites as desfuoylceftiofur-protein conjugates, are converted into desfuoylceftiofuracetamide (DCA), which was determined by HPLC. In brief, 1 mL of serum or lochia, or 0.5 g of endometrium, caruncles, or cotyledons were mixed with 5 mL of 50 mM potassium tetraborate (Merck, Darmstadt, Germany) at pH 9.0, containing 0.5 M sodium chloride (Merck) and 130 mM dithioerythritol (Sigma-Aldrich, Zwijndrecht, the Netherlands), and incubated at 50°C for 15 min with intermittent mixing at 5-min intervals. Following this reduction, 5 mL of 0.1 M ammonium acetate (J.T. Baker, Deventer, the Netherlands) containing 0.2 M iodoacetamide (Sigma-Aldrich) was added, mixed, and the incubation was continued in the dark at ambient temperature for 30 min under gentle agitation at 500 rpm. Homogenized lochia, caruncles, cotyledons, and endometrium were acidified to pH 3 with 0.08 mL of 17% phosphoric acid. After centrifugation at $4,000 \times g$ for

Figure 1. Mean concentrations ($\pm$ SEM) of desfuroylceftiofuracetamide in serum after 3 subcutaneous administrations of 1 mg of ceftiofur equivalents/kg of BW of ceftiofur hydrochloride sterile suspension at 0, 24, and 48 h.

Figure 2. Mean concentrations ($\pm$ SEM) of desfuroylceftiofuracetamide in endometrial tissue after 3 subcutaneous administrations of 1 mg of ceftiofur equivalents/kg of BW of ceftiofur hydrochloride sterile suspension at 0, 24, and 48 h.

30 min at 5°C, the pH of the supernatant was adjusted to pH 5 with approximately 0.1 mL of 5 M sodium hydroxide and was then passed through a Bond Elut C₁₈-solid-phase extraction (SPE) cartridge (1 g; Varian, Bergen op Zoom, the Netherlands). Cartridges were washed with 5 mL of 0.1 M ammonium acetate and 5 mL of 2% (vol/vol) acetic acid, and then eluted with 5 mL of a mixture of acetonitrile (J.T. Baker) and 2% (vol/vol) acetic acid (Merck) at a ratio of 2:8 (vol/vol). Before their use, columns were activated with 5 mL of methanol and conditioned with 5 mL of 0.1 M ammonium acetate solution (J.T. Baker).

Analyte-containing C₁₈-SPE eluates were passed over a cation exchanger SCX SPE column (100 mg; Varian), which was then washed with 1 mL of methanol. Retained DCA was eluted with 1.0 mL of a mixture of 1.0 M ammonium acetate and acetonitrile at a ratio of 85:15 (vol/vol). Before their use, the SCX cartridges were activated with 0.2 mL of methanol and conditioned with 2 mL of 2% (vol/vol) acetic acid (Merck) in water, respectively.

The HPLC analysis of 50-µL samples was carried out on a 3-µm C₁₈ column (50 × 4.6 mm; Phenomenex, Torrance, CA) and a 3-µm phenyl-hexyl (50 × 4.6 mm; Phenomenex) column that were connected in line. Elution of analytes was performed using a binary linear gradient of 10 mM ammonium acetate at pH 6.8 (eluent A) and acetonitrile (eluent B) at a flow rate of 1.0 mL/min as follows: 99% A (vol/vol) for 1.9 min, to 92% A (vol/vol) in 0.1 min, to 82% A (vol/vol) in 12 min, and to 0% A for 7 min. The eluate was monitored at 266 nm. Sample series were analyzed with a repetitive analysis time of 30 min per sample. Limit of quantification of the method was 0.1 µg/mL of ceftiofur for lochia and serum and 0.1 µg/g of ceftiofur for caruncles, cotyledons, and endometrium. Standards were prepared from

blank corresponding biological materials spiked at 8 different concentration points between 0.1 and 10 µg/mL (µg/g) of ceftiofur (Pfizer Animal Health, Puurs, Belgium) and then processed and analyzed simultaneously and in an identical way as the laboratory samples. Standards were used to obtain a standard curve following linear regression analysis. In fact, the standard curve was used as a control of the quality of the analysis procedure.

Interpretation and Analyses

When concentrations of DCA were greater than the standard of 10 µg/mL (µg/g), these samples were reanalyzed, and when values were reproducible, they were regarded as outliers. In these cases, values were excluded from analyses.

Concentrations of DCA of ≥ 0.5 µg/mL or ≥ 0.5 µg/g and of ≥ 0.125 µg/mL or ≥ 0.125 µg/g are relevant for the potential efficiency of a treatment against *E. coli* and *A. pyogenes*, and *F. necrophorum* and *P. melaninogenicus*, respectively (Cervantes et al., 1993; Salmon et al., 1996; Sheldon et al., 2004). Therefore, these concentrations were regarded as threshold values.

Correlation of time after C1, temperature, and concentration of DCA in serum with concentrations of DCA in serum, endometrium, caruncles, cotyledons, and lochia was tested by using Spearman's rank correlation for nonparametric variables (SPSS for Windows 12.0, SPSS Inc., Munich, Germany). Samples collected before the first of 3 injections of ceftiofur hydrochloride were excluded from these analyses. Level of significance was set at $\alpha = 0.05$.

RESULTS

Described analytical methods were found to be suitable for determining DCA in described bovine materials

Table 1. Numbers of samples with concentrations of desfuryleftiofuracetamide (DCA) <0.5 and 0.125 µg/mL (serum and lochia) or 0.5 and 0.125 µg/g (endometrium, caruncles, and cotyledons) after 3 subcutaneous administrations (24 h apart) of 1 mg of ceftiofur equivalents per kg of BW as ceftiofur hydrochloride sterile suspension

Tissue	Concentration of DCA ²	Time of sampling after first of 3 injections, ¹ h												
		0	2	4	12	24	26	28	36	48	50	52	60	72
Serum	<0.5	6/6	2/6	—	—	2/6	—	—	—	3/6	—	—	—	—
	<0.125	6/6	—	—	—	—	—	—	—	—	—	—	—	—
Lochia	<0.5	6/6	4/6	4/6	4/6	5/6	5/6	2/6	3/6	2/5	4/5	3/5	1/5	2/5
	<0.125	6/6	4/6	—	—	—	1/6	1/6	—	—	1/5	—	—	1/5
Endometrium	<0.5	6/6	4/6	—	—	2/6	—	1/6	—	1/5	—	—	—	—
	<0.125	6/6	1/6	—	—	1/6	—	—	—	1/5	—	—	—	—
Caruncle	<0.5	6/6	6/6	5/6	—	1/6	—	—	—	1/6	—	—	1/6	—
	<0.125	6/6	1/6	—	—	—	—	—	—	—	—	—	—	—
Cotyledon	<0.5	6/6	5/6	4/6	—	—	—	—	—	2/6	—	1/6	1/5	1/3
	<0.125	6/6	1/6	—	—	—	—	—	—	—	—	—	—	—

¹Fields without numbers indicate that all samples exceeded reference concentrations.

²For serum and lochia, concentrations are in µg/mL; for endometrium, caruncle, and cotyledon tissues, concentrations are in µg/g.

because the coefficients of determination of standard curves were better than 0.9994 ($n = 12$). Only once was this coefficient less than <0.0994 (0.991 for endometrium). The interday variance of the slopes of the curves did not vary more than 15% in most cases, indicating acceptable reproducibility of the test results. Outliers were found in 4 (2 in serum, 1 in caruncular tissue, and 1 in lochia) of 381 analyzed samples, and those values were excluded from further analyses.

Serum

A total of 78 samples were analyzed for concentrations of DCA in serum. Mean concentration reached 0.64 µg/mL at 2 h and remained above the threshold value of 0.5 µg/mL during the entire observation period. A maximum mean concentration during the collection period before a subsequent administration of ceftiofur was reached at 12 (1.10 µg/mL), 4 (1.18 µg/mL), and 4 (1.66 µg/mL) h after C1, C2, and C3, respectively (Figure 1).

Concentrations increased in all cows above the threshold value of 0.125 µg/mL by 2 h and above 0.5 µg/mL by 4 h after the initial injection of ceftiofur hydrochloride. In 2 samples, however, concentrations decreased to <0.5 µg/mL at 24 h (i.e., immediately before C2), and in 3 samples immediately before C3 (Table 1). Maximum concentration in a single sample was 3.25 µg/mL.

Endometrial Tissue

Endometrial tissue was analyzed in 78 samples. Within 4 h after C1 and until the end of the observation period, mean concentration of DCA exceeded the

threshold value of 0.5 µg/g. Mean concentration of 0.125 µg/g was reached at 2 h. Maximum mean concentrations in endometrial tissue were found at 12 h (1.35 µg/g), 2 h (1.39 µg/g), and 2 h (2.02 µg/g) after C1, C2, and C3, respectively (Figure 2). After 2 h, concentrations decreased to <0.5 µg/g were found in 4 samples and <0.125 µg/g in 2 samples (Table 1).

Caruncular Tissue

Mean concentrations of DCA above the threshold value of 0.125 µg/g were reached at 2 h, and greater than 0.5 µg/g at 12 h after C1. Mean concentration remained above 0.5 µg/g until the end of the observation period. A maximum in mean concentrations was found at 12 h (1.03 µg/g), 12 h (1.95 µg/g), and 4 h (2.36 µg/g) after C1, C2, and C3, respectively (Figure 3). In 1 cow, concentrations of DCA decreased to <0.5 µg/g at 24 and 48 h (i.e., immediately before C2 and C3, and again at 60 h).

Cotyledonal Tissue

Cotyledonal tissue could not be obtained from 1 cow at 60 and 72 h and could not be obtained from 2 other cows at 72 h. Mean concentrations of DCA exceeded 0.125 µg/g at 2 h and 0.5 µg/g at 12 h after C1 and until the end of the observation period. A maximum in mean concentrations of DCA in cotyledonal tissue was found at 24 h (1.75 µg/g), 12 h (2.14 µg/g), and 2 h (2.56 µg/g) after C1, C2, and C3, respectively (Figure 4). After 4 h, concentrations decreased to <0.5 µg/g were found in 1 cow at 48 h and 52 h to 72 h after C1, and in another cow at 48 h after C1 (Table 1).

Figure 3. Mean concentrations ($\pm$ SEM) of desfuroylceftiofuracetamide in caruncular tissue after 3 subcutaneous administrations of 1 mg of ceftiofur equivalents/kg of BW of ceftiofur hydrochloride sterile suspension at 0, 24, and 48 h.

Figure 5. Mean concentrations ($\pm$ SEM) of desfuroylceftiofuracetamide in lochia after 3 subcutaneous administrations of 1 mg of ceftiofur equivalents/kg of BW of ceftiofur hydrochloride sterile suspension at 0, 24, and 48 h.

Lochia

In 1 cow, lochia could not be obtained at 48 and 72 h after C1. Mean concentration of DCA exceeded threshold values of 0.125 $\mu\text{g/mL}$ at 2 h and of 0.5 $\mu\text{g/mL}$ at 12 h. Mean concentration remained $>0.5 \mu\text{g/mL}$ until the end of the observation period (Figure 5). A maximum in mean concentrations was reached at 12 h (0.87 $\mu\text{g/mL}$), 2 h (1.32 $\mu\text{g/mL}$), and 4 h (2.33 $\mu\text{g/mL}$) after C1, C2, and C3, respectively (Figure 5). At none of the sampling points, however, did all cows reach concentrations $>0.5 \mu\text{g/mL}$. Concentrations $<0.125 \mu\text{g/mL}$ were found after 2 h in 4 samples (Table 1).

Temperature

Mean rectal temperatures are shown in Figure 6. After an initial increase at 4 h (39.3°C), temperature declined moderately to 38.9°C at 24 h. Thereafter, mean

temperatures increased to a maximum of 40.1°C at 52 h and decreased to a mean rectal temperature of 39.3°C at the end of the observation period.

Correlations

A significant positive correlation of time after the first of 3 injections was found with concentrations of DCA in uterine tissues, cotyledons, and lochia, but not of time with concentration of DCA in serum. Correlations of rectal temperature with concentration of DCA in serum, endometrial, and caruncular tissues, and in lochia, also were positive, but not of temperature with concentration of DCA in cotyledonal tissue. Correlations of concentrations of DCA in serum with concentrations in uterine tissues and cotyledons were positive, but not with concentrations in lochia (Table 2).

Figure 4. Mean concentrations ($\pm$ SEM) of desfuroylceftiofuracetamide in cotyledonal tissue after 3 subcutaneous administrations of 1 mg of ceftiofur equivalents/kg of BW of ceftiofur hydrochloride sterile suspension at 0, 24, and 48 h.

Figure 6. Mean temperatures ($\pm$ SEM) after 3 subcutaneous administrations of 1 mg of ceftiofur equivalents/kg of BW of ceftiofur hydrochloride sterile suspension at 0, 24, and 48 h.

Table 2. Correlations of time after the first treatment with ceftiofur hydrochloride, rectal temperature, and concentrations of desfuroylceftiofuracetamide (DCA) in serum with concentrations of DCA in serum, endometrium, caruncles, cotyledons, and lochia after 3 subcutaneous administrations (24 h apart) of 1 mg of ceftiofur equivalents per kg of BW as ceftiofur hydrochloride sterile suspension

Item	Serum	Endometrium	Caruncles	Cotyledons	Lochia
No. of samples	72	70	71	68	66
Time after initial injection	0.19	0.38*	0.64*	0.30*	0.29*
Temperature	0.30*	0.30*	0.42*	0.18	0.28*
Serum	1.00	0.50*	0.44*	0.27*	-0.11

* $P < 0.05$.

DISCUSSION

The objective of the present study was to determine concentrations of ceftiofur derivatives in serum, endometrium, caruncles, cotyledons, and lochia of cows having RFM after 3 subcutaneous injections with 1 mg of ceftiofur equivalents/kg of BW as ceftiofur hydrochloride sterile suspension. This is the first study describing pharmacokinetics of ceftiofur hydrochloride in cows treated for RFM.

Pharmacokinetics of ceftiofur have been described for several animals, including cattle (Brown et al., 2000; Okker et al., 2002), pigs (Brown et al., 1999), mares (Jonker, 1997), sheep (Craigmill et al., 1997), goats (Courtin et al., 1997), and llama and alpaca (Drew et al., 2004). In most reports, however, pharmacokinetics were determined for ceftiofur in plasma. Only in a few studies were concentrations of ceftiofur examined in uterine tissues. After intramuscular administration of 2 mg/kg of ceftiofur to 4 mares, Jonker (1997) found mean concentrations of 1.23 $\mu\text{g/g}$ of DCA in endometrial tissue. Okker et al. (2002) have demonstrated that subcutaneous administration of 1 mg/kg of BW of ceftiofur hydrochloride to 4 healthy postpartum cows resulted in mean concentrations of ceftiofur derivatives in plasma and uterine tissues that exceed reported MIC_{90} value of 0.5 $\mu\text{g/mL}$ for *E. coli* and of 0.125 $\mu\text{g/mL}$ for *A. pyogenes*, *F. necrophorum*, and *P. melaninogenicus* (Salmon et al., 1996; Sheldon et al., 2004). The predominant bacterial pathogen for acute metritis is *E. coli*, and therefore, the focus of this discussion is on the threshold value of 0.5 $\mu\text{g/mL}$. The threshold value of 0.125 $\mu\text{g/mL}$, however, which is relevant for *A. pyogenes*, *F. necrophorum*, and *P. melaninogenicus*, was exceeded in all tissues at 2 h. Mean concentrations remained greater than this value for the entire observation period.

In the present study, increased mean concentration of ceftiofur derivatives in serum to a first maximum was comparable with observations of Okker et al. (2002), who found a maximum of 2.85 $\mu\text{g/mL}$ in plasma 2 h after administration. Mean concentrations of ceftiofur derivatives exceeded 0.5 $\mu\text{g/g}$ in uterine tissues and

cotyledons by 4 h (endometrium) and 12 h (caruncles and cotyledons), respectively, after the initial treatment. The increase of concentrations in endometrial and caruncular tissues was similar to findings of Okker et al. (2002), who described peaks at 5 h (endometrium) and 11.5 h (caruncles) after treatment. In endometrial tissue, however, first maximum concentration of 1.35 $\mu\text{g/g}$ was <2.25 $\mu\text{g/g}$ reported by Okker et al. (2002). For clinical efficacy of this treatment, it might be of interest that concentrations of ceftiofur derivatives in endometrium and caruncles increased to a maximum (2.02 and 2.36 $\mu\text{g/g}$, respectively) after the third daily injection.

Mean concentrations of ceftiofur derivatives in serum, uterine tissues, and cotyledons remained greater than reported MIC_{90} values for common uterine pathogens of 0.5 $\mu\text{g/mL}$ (Sheldon et al., 2004) during the entire observation period. In single samples, however, mainly immediately before the second and third injection of ceftiofur hydrochloride, concentrations decreased to <0.5 $\mu\text{g/mL}$. Thus, in few instances, suboptimal concentrations during the recommended treatment period of 5 d might occur. It is not possible to describe the duration of concentrations below 0.5 $\mu\text{g/mL}$ because samples were collected at 12 and 24 h after each injection. Also, it is not clear whether this finding of suboptimal concentrations in our study is practically or pharmacologically relevant.

Tissue concentrations of β -lactams are known to underestimate drug concentrations in the extracellular fluid. When tissues are homogenized for analysis the intracellular fluid is liberated and dilutes the extracellular fluid. This is especially true for β -lactams that do not penetrate cells very well because of their limited lipid solubility (Brown et al., 1995). Further research is required to evaluate whether implementing a shorter interval between injections or using a larger dose of 2.2 mg of ceftiofur hydrochloride/kg of BW, which is approved in the United States, would result in different pharmacokinetic patterns.

For lochia, results were more heterogeneous. After the initial daily injection with ceftiofur, the increase of

mean concentration was similar to those reported by Okker et al. (2002), who found a maximum of 0.98 µg/mL at 5 h. After the second daily injection of ceftiofur hydrochloride, concentrations were very inconsistent among cows. At all collection periods, concentrations of ceftiofur derivatives were <0.5 µg/mL in some cows. This might be explained by variable amounts of lochia in the uterine cavity and by unknown interactions of ceftiofur binding to organic debris. It is questionable whether elevated concentrations of ceftiofur in lochia are essential for effective treatment. It is probably more important to achieve effective concentrations in uterine tissues at the site of inflammation and infection.

In serum, uterine tissues, cotyledons, and lochia, concentrations of ceftiofur derivatives showed a wave-like pattern with reduced concentrations 24 h after each injection of ceftiofur hydrochloride. In uterine tissues, cotyledons, and lochia, concentrations increased during the observation period. This indicates an accumulation of ceftiofur derivatives in inflamed tissues in cows with RFM. This confirms results of Clarke et al. (1996) who found greater concentrations of ceftiofur in implanted tissue chambers in calves inoculated with *Pasteurella haemolytica* than in noninfected chambers. The study by Okker et al. (2002) was performed on healthy cows over a period of 24 h after a single injection of ceftiofur hydrochloride. Therefore, it is not clear whether this accumulation is specific to cows with uterine infections or also prevalent in healthy cows.

Several plausible interpretations exist for the positive correlation of rectal temperature with concentrations of ceftiofur derivatives in serum, endometrial, and caruncular tissues. One is that temperature increased during the study period because of intrauterine manipulations. This hypothesis is confirmed by an increased temperature 2 and 4 h after sampling. A second explanation is that with elevated concentration of ceftiofur derivatives in serum and uterine tissues, the bactericide potential of the antimicrobial increases and releases bacterial and probably pyretic toxins. A third interpretation is that the increase of temperature is independent from sampling and concentrations of ceftiofur derivatives and occurs to a large extent in cows having RFM regardless of an antimicrobial therapy. Increased rectal temperature has been described for RFM cows treated systemically with ceftiofur hydrochloride as well as for cows treated with intrauterine antibiotics (Drillich et al., 2006a,b). In another study by Risco and Hernandez (2003), however, treatment of RFM cows with 2.2 mg of ceftiofur/kg of BW was beneficial for prevention of metritis (i.e., fever and fetid discharge).

This study demonstrated that in cows with RFM, mean concentrations of ceftiofur derivatives exceeded

reported MIC₉₀ values for *E. coli*, a common uterine pathogen in serum and endometrium by 2 and 4 h, respectively, in caruncular and cotyledonal tissue, and in lochia by 12 h after injecting 1 mg of ceftiofur equivalents/kg of BW as ceftiofur hydrochloride sterile suspension. Mean concentrations exceeded MIC₉₀ values for *A. pyogenes*, *F. necrophorum*, and *P. melaninogenicus* in all tissues by 2 h. After a second and third daily injection of ceftiofur hydrochloride, mean concentrations of ceftiofur derivatives did not decrease below MIC₉₀ values until after the end of the study period of 72 h. Only in single samples, especially 24 h after treatment, did concentrations decrease temporarily below MIC₉₀ values.

ACKNOWLEDGMENTS

Ceftiofur sodium (898 mg/g of active substance) for HPLC analysis was a kind gift from Pfizer Animal Health, Puurs, Belgium.

REFERENCES

- Brown, S. A., S. T. Chester, A. K. Speedy, V. L. Hubbard, J. K. Callahan, P. J. Hamlow, B. Hibbard, and E. J. Robb. 2000. Comparison of plasma pharmacokinetics and bioequivalence of ceftiofur sodium in cattle after a single intramuscular or subcutaneous injection. *J. Vet. Pharmacol. Ther.* 23:273–280.
- Brown, S. A., N. L. DeLeeuw, G. L. Stahl, and R. D. Roof. 1995. Concentrations of ceftiofur and metabolites in mouse lung perfused or non perfused in situ compared to tilmicosin. *J. Vet. Pharmacol. Ther.* 18:385–387.
- Brown, S. A., B. J. Hanson, A. Mignot, L. Millerioux, P. J. Hamlow, V. L. Hubbard, J. K. Callahan, and F. M. Kausche. 1999. Comparison of plasma pharmacokinetics and bioavailability of ceftiofur sodium and ceftiofur hydrochloride in pigs after a single intramuscular injection. *J. Vet. Pharmacol. Ther.* 22:35–40.
- Cervantes, C. C., M. P. Brown, R. Gronwall, and K. Merritt. 1993. Pharmacokinetics and concentrations of ceftiofur sodium in body fluids and endometrium after repeated intramuscular injections in mares. *Am. J. Vet. Res.* 54:573–575.
- Chenault, J. R., J. F. McAllister, S. T. J. Chester, K. J. Dame, F. M. Kausche, and E. J. Robb. 2004. Efficacy of ceftiofur hydrochloride sterile suspension administered parenterally for the treatment of acute postpartum metritis in dairy cows. *J. Am. Vet. Med. Assoc.* 224:1634–1639.
- Clarke, C. R., S. A. Brown, R. N. Streeter, J. M. Clarke, P. J. Hamlow, J. K. Callahan, V. L. Hubbard, A. K. Speedy, and G. E. Burrows. 1996. Penetration of parenterally administered ceftiofur into sterile vs. *Pasteurella haemolytica*-infected tissue chambers in cattle. *J. Vet. Pharmacol. Ther.* 19:376–381.
- Courtin, F., A. L. Craigmill, S. E. Wetzlich, C. R. Gustafson, and T. S. Arndt. 1997. Pharmacokinetics of ceftiofur and metabolites after single intravenous and intramuscular administration and multiple intramuscular administrations of ceftiofur sodium to dairy goats. *J. Vet. Pharmacol. Ther.* 20:368–373.
- Craigmill, A. L., S. A. Brown, S. E. Wetzlich, C. R. Gustafson, and T. S. Arndt. 1997. Pharmacokinetics of ceftiofur and metabolites after single intravenous and intramuscular administration and multiple intramuscular administrations of ceftiofur sodium to sheep. *J. Vet. Pharmacol. Ther.* 20:139–144.
- Drew, M. L., L. Johnson, D. Pugh, C. B. Navarre, I. T. Taylor, and A. L. Craigmill. 2004. Pharmacokinetics of ceftiofur in llamas and alpacas. *J. Vet. Pharmacol. Ther.* 27:13–20.

- Drillich, M., O. Beetz, A. Pfutzner, M. Sabin, H. J. Sabin, P. Kutzer, H. Nattermann, and W. Heuwieser. 2001. Evaluation of a systemic antibiotic treatment of toxic puerperal metritis in dairy cows. *J. Dairy Sci.* 84:2010–2017.
- Drillich, M., M. Mahlstedt, U. Reichert, B. A. Tenhagen, and W. Heuwieser. 2006a. Strategies to improve the therapy of retained fetal membranes in dairy cows. *J. Dairy Sci.* 89:627–635.
- Drillich, M., A. Pfutzner, H. J. Sabin, M. Sabin, and W. Heuwieser. 2003. Comparison of two protocols for the treatment of retained fetal membranes in dairy cattle. *Theriogenology* 59:951–960.
- Drillich, M., U. Reichert, M. Mahlstedt, and W. Heuwieser. 2006b. Comparison of two strategies for a systemic antibiotic treatment of dairy cows with retained fetal membranes: Preventive vs. selective treatment. *J. Dairy Sci.* 89:1502–1508.
- Hornish, R. E., and S. F. Kotarski. 2002. Cephalosporins in veterinary medicine—Ceftiofur use in food animals. *Curr. Top. Med. Chem.* 2:717–731.
- Jonker, F. H. 1997. Secretion of ceftiofur in equine endometrium after parenteral administration. *J. Vet. Pharmacol. Ther.* 20(Suppl.):37.
- Okker, H., E. J. Schmitt, P. L. Vos, P. Scherpenisse, A. A. Bergenwerff, and F. H. Jonker. 2002. Pharmacokinetics of ceftiofur in plasma and uterine secretions and tissues after subcutaneous postpartum administration in lactating dairy cows. *J. Vet. Pharmacol. Ther.* 25:33–38.
- Olson, S. C., M. G. Beconi-Barker, E. B. Smith, R. A. Martin, T. J. Vidmar, and L. D. Adams. 1998. In vitro metabolism of ceftiofur in bovine tissues. *J. Vet. Pharmacol. Ther.* 21:112–120.
- Risco, C. A., and J. Hernandez. 2003. Comparison of ceftiofur hydrochloride and estradiol cypionate for metritis prevention and reproductive performance in dairy cows affected with retained fetal membranes. *Theriogenology* 60:47–58.
- Salmon, S. A., J. L. Watts, and R. J. Yancey. 1996. In vitro activity of ceftiofur and its primary metabolite, desfuroylceftiofur, against organisms of veterinary importance. *J. Vet. Diagn. Invest.* 8:332–336.
- Sheldon, I. M., M. Bushnell, J. Montgomery, and A. N. Rycroft. 2004. Minimum inhibitory concentrations of some antimicrobial drugs against bacteria causing uterine infections in cattle. *Vet. Rec.* 155:383–387.
- Sheldon, I. M., and H. Dobson. 2004. Postpartum uterine health in cattle. *Anim. Reprod. Sci.* 82–83:295–306.
- Smith, B. I., G. A. Donovan, C. Risco, R. Littell, C. Young, L. H. Stanker, and J. Elliott. 1998. Comparison of various antibiotic treatments for cows diagnosed with toxic puerperal metritis. *J. Dairy Sci.* 81:1555–1562.
- Zhou, C., J. F. Boucher, K. J. Dame, M. Moreira, R. Graham, J. Nantel, S. Zuidhof, L. Arfi, R. Flores, G. Neubauer, and J. Olson. 2001. Multilocation trial of ceftiofur for treatment of postpartum cows with fever. *J. Am. Vet. Med. Assoc.* 219:805–808.