



Determination of ceftiofur derivatives in serum, endometrial tissue, and lochia in puerperal dairy cows after subcutaneous administration of ceftiofur crystalline free acid

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ABSTRACT

Puerperal uterine infections are often associated with decreased reproductive performance in dairy cows. Routine treatment protocols include the systemic administration of antibiotics. Antibiotic drugs, however, should be administered daily over at least 5 d. The objective of this study was to determine concentrations of ceftiofur derivatives in serum, endometrial tissue, and lochia after subcutaneous administration of ceftiofur crystalline free acid in 6 clinically healthy puerperal dairy cows with normal parturition. Samples were taken immediately before treatment, 2 h after, and then every 24 h over a 7-d period. Concentrations of ceftiofur derivatives were quantified using an HPLC assay. In serum and endometrial tissue, ceftiofur derivatives could be detected above the reported minimum drug concentrations required to inhibit relevant pathogens such as *Escherichia coli* and *Arcanobacterium pyogenes* over a 7-d period. Concentrations of desfuoylceftiofuracetamide at 5 d after administration of ceftiofur crystalline free acid were $1.21 \pm 0.61 \mu\text{g/mL}$ in serum, $0.86 \pm 0.61 \mu\text{g/mg}$ in endometrial tissue, and $0.96 \pm 1.15 \mu\text{g/mL}$ in lochia. In lochia, mean concentrations of ceftiofur derivatives also remained above the minimal inhibitory concentration of relevant pathogens, but showed greater variations between cows.

Key words: ceftiofur crystalline free acid, minimum inhibitory concentration, dairy cow, puerperal endometritis

INTRODUCTION

Puerperal uterine infections such as metritis and endometritis are common problems in high-producing dairy cows (Galvão et al., 2009) and often associated with decreased reproductive performance (Gilbert et

al., 2005). Uterine function can be compromised by bacterial contamination of the uterus after parturition. Relevant pathogens often persist and lead to uterine infections, which can cause infertility (Sheldon et al., 2006). The most common bacteria found in postpartum uterine diseases in cows are *Escherichia coli*, *Arcanobacterium pyogenes*, and aerobic species such as *Fusobacterium necrophorum* and *Prevotella melaninogenica* (Sheldon and Dobson, 2004). A common method to reduce the effect of uterine infection on fertility is intrauterine or systemic treatment with antibiotics (Galvão et al., 2009). The use of a local antibiotic treatment, however, is controversial (Drillich et al., 2001). Adverse interactions of the antibiotic drug and the uterine environment, irritation of the endometrium by the antibiotic drug itself or the carrier substance, and a limited efficacy of the antibiotics within the inflamed tissue make local treatment with antibiotics questionable (Paisley et al., 1986). In the last decade, efficacy of intervention strategies based on the systemic application of broad-spectrum antibiotics has been demonstrated repeatedly (Smith et al., 1998; Drillich et al., 2006a; Galvão et al., 2009).

Ceftiofur is a third-generation cephalosporin, an important class of antimicrobial drugs used in veterinary medicine (Hornish and Kotarski, 2002). Good efficacy of ceftiofur for treatment of retained fetal membranes (RFM; Drillich et al., 2006a, b) and acute puerperal metritis (APM; Drillich et al., 2001) has been demonstrated. Furthermore, intrauterine infusion of ceftiofur hydrochloride positively affected uterine health in dairy cows but failed to decrease the incidence of subclinical endometritis or improve reproductive performance (Galvão et al., 2009). Ceftiofur is a highly effective drug against almost all gram-positive and gram-negative pathogens (Salmon et al., 1996; Haggett and Wilson, 2008). In the liver, it is hydrolyzed to desfuoylceftiofuracetamide (DCA), an active metabolite that is protected from fast renal elimination through protein binding (Brown et al., 1991). Desfuoylceftio-

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furacetamide reaches maximum plasma concentrations 2 h after i.m. and 3 h after s.c. administration with a half-life of 9.65 ± 1.97 h in calves (Brown et al., 2000). Science-based evidence shows that concentrations of DCA in the endometrium of puerperal cows exceed the reported MIC for *E. coli* (0.5 $\mu\text{g}/\text{mL}$) and *A. pyogenes* (0.125 $\mu\text{g}/\text{mL}$) after a single (Okker et al., 2002) or 3 consecutive (Drillich et al., 2006b) s.c. administrations, respectively. In single samples, however, the concentration decreased below the MIC for *E. coli* (Drillich et al., 2006b). Similarly, concentrations of ceftiofur remained above the reported MIC for relevant equine pathogens such as *Streptococcus zooepidemicus* in both serum and endometrial tissue until 24 h after treatment of healthy mares (Witte et al., 2010). In cows with signs of clinical endometritis diagnosed between 21 and 28 d postpartum, however, the systemic treatment with ceftiofur on 3 consecutive days had the same effect as a treatment protocol using 2 doses of cloprostenol in a 14-d interval (Kaufmann et al., 2010). In the first 3 wk after parturition, however, infections of the uterus are seldom restricted to the endometrium. Therefore, parenteral administration of antibiotics is recommended to provide adequate drug concentrations in all uterine tissues (Bretzlaff et al., 1983).

Cephalosporins have a clinical efficacy, which depends on the time during which drug concentrations remain above the MIC of the pathogen. In a study of Sheldon et al. (2004), the MIC of commonly used antibiotics for principal bacteria associated with uterine infections were evaluated. In the present study, we refer to the MIC values of *E. coli* ($\text{MIC}_{90} = 0.5 \mu\text{g}/\text{mL}$), *A. pyogenes* ($\text{MIC}_{90} = 0.125 \mu\text{g}/\text{mL}$), *F. necrophorum* ($\text{MIC}_{90} = 0.125 \mu\text{g}/\text{mL}$), and *P. melaninogenica* ($\text{MIC}_{90} = 0.125 \mu\text{g}/\text{mL}$; Sheldon et al., 2004). It is, therefore, important to demonstrate that levels of the active metabolite are maintained above the MIC of target pathogens for an adequate period (Drillich et al., 2001, 2006b; Chenault et al., 2004). In the United States and Europe, ceftiofur (Excenel RTU, Pfizer Animal Health, New York, NY and Pfizer Animal Health, Berlin, Germany, respectively) has been approved for the treatment of APM for 5 consecutive days. Anecdotal evidence from the field, however, suggests that on average only 2 to 3 treatments are administered for therapy of feverish metritis. It is unknown if a shortened time above MIC causes suboptimal cure rates, poses a risk for subclinical endometritis, and reduces reproductive performance. Therefore, an intervention based on a single injection of a long-acting formulation to administer an effective treatment of APM would be advantageous.

Ceftiofur crystalline free acid (CCFA, Naxcel, Pfizer Animal Health, Berlin, Germany) is a sustained-release formulation of ceftiofur that is approved for acute

bovine interdigital necrobacillosis. After s.c. administration at the base of the ear, it sustained effective serum concentrations over a 7.6-d period (Hibbard et al., 2004).

The objective of the present study was to determine concentrations of DCA in puerperal dairy cows after a single s.c. administration of CCFA over a period of 7 d. The hypothesis to be tested was that the concentration of DCA determined in serum, endometrial tissue, and lochia after a single s.c. injection of 6.6 mg/kg of BW of CCFA at the base of the ear exceeded the reported MIC for *E. coli* and *A. pyogenes*, *F. necrophorum*, and *P. melaninogenica* for a 7-d period.

MATERIALS AND METHODS

Experimental Animals and Design

The study was conducted between January and April 2010 at the Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Germany. A total of 6 healthy Holstein-Friesian cows was included in the study (5 cows expelled their fetal membranes within 12 h, 1 cow had retained fetal membranes). Cows were housed at the Clinic for Animal Reproduction in a tie-stall barn with straw bedding from 14 ± 10 d before parturition until the end of the study. Parturition occurred spontaneously. During 14 d before parturition and until the end of the study, none of the cows enrolled received antibiotics or antiinflammatory drugs other than CCFA.

The study period was defined as the time from collection of the baseline samples until collection of the last samples; that is, 7 d after administration of CCFA. Twelve to 24 h after calving, cows received a single dose of 6.6 mg/kg of BW of CCFA s.c. at the base of the ear. Immediately before administration, baseline samples of serum, endometrial tissue, and lochia were collected. Additional blood, tissue, and lochia samples were taken within $2 \text{ h} \pm 30 \text{ min}$ after administration of CCFA and then every $24 \text{ h} \pm 1 \text{ h}$ for 7 d.

Methods of Sampling

Before taking the samples, rectal temperature was measured with a digital thermometer (Microlife AG, Heerbrugg, Switzerland). Blood samples were collected from coccygeal vessels using sterile vacuum tubes (Venject II, Termumo Europe N.V., Leuven, Belgium). Before further processing of the blood samples, BHBA was detected using an electronic BHBA measuring system (Precision Xtra, Abbott, Abingdon, UK), as described previously (Iwersen et al., 2009). Within 30 min after sampling, blood samples were centrifuged

at $3,500 \times g$ for 8 min at room temperature and serum stored at -20°C until further analysis.

Before collection of samples from the uterus, the perineum was washed 3 times with a disinfectant soap and dried with paper towels. Biopsy samples were taken from the base of either uterine horn with a Kevorkian biopsy forceps (WDT, Garbsen, Germany), as described for horses (modified after Ricketts, 1975). In brief, the biopsy forceps was manually introduced through the spread vulva into the vagina and into or through the cervical canal. The hand was removed and the biopsy forceps was placed at the base of one uterine horn through rectal control. A sample of uterine tissue (approximately 0.15 g) was collected under transrectal control. The collected tissue was stored in sterile plastic tubes (Eppendorf, Hamburg, Germany) at -20°C until further analysis.

Finally, one sterile-gloved hand or an insemination pipette was introduced through the vagina into the uterine lumen and at least 3 mL of lochia was collected. Within 30 min after collection, lochia samples were stored in 10-mL plastic tubes (Eppendorf) at -20°C until further analysis.

Additionally, from one healthy, nontreated cow, 100 mL of serum, 10 g of endometrial tissue, and 100 mL of lochia were collected within 24 h after calving as blank material for HPLC analysis.

Analytical Methods

Concentrations of ceftiofur (molecular weight = 523.56 g/mol) residues were quantified in serum, endometrial tissue, and lochia samples using an HPLC assay as described by Drillich et al. (2006b) and Witte et al. (2010). In this method, residues of ceftiofur were converted into DCA (molecular weight = 486.54 g/mol), which was determined by HPLC.

Briefly, 1.0 mL of serum or lochia or 0.1 g of endometrium was mixed with 5.0 mL of dithioerythritol solution (20 mg/mL dithioerythritol solution in 50 mM potassium tetra borate buffer containing 0.5 M sodium chloride) at pH 9. Endometrial tissue was homogenized using an Ultra-Turrax homogenizer (IKA, Staufen, Germany) for 30 s. All following steps in sample treatment were carried out as described in Drillich et al. (2006b). For HPLC analysis, 50 μL injections were made on an Inertsil ODS-2 column (200 \times 3 mm, Varian Medical Systems, Houten, the Netherlands). A gradient program was run by an HPLC pump (Merck-Hitachi, Tokyo, Japan) mixing 10 mM ammonium acetate in water (eluent A) and acetonitrile (eluent B) at a flow rate of 0.40 mL/min. The gradient started with 99% A for 1.9 min, to 92% A in 0.1 min, to 82% A in 12 min,

to 0% A in 1 min and held at 0% A for 7 min. Then, the column was equilibrated for 8 min, resulting in a total run time of 30 min. The eluate of the column was monitored using a UV detector (Applied Biosystems, Foster City, CA) operated at 266 nm. Chromatograms were recorded onto a computer running ChromQuest version 2.51 (Thermo, Breda, the Netherlands). Standards for quantification were prepared by processing blank serum spiked with ceftiofur in a range of 0.05 to 10.0 $\mu\text{g}/\text{mL}$. These samples were processed together at the same time with real study samples.

For analysis of the endometrial tissue an additional liquid chromatography tandem MS was performed. In brief, 10- μL injections were made into an HPLC system consisting of 2 HPLC pumps (PE200 series, Perkin-Elmer, Waltham, MA), an autosampler (CTC Analytics, Zwingen, Switzerland), and an API4000 MS detector with an electrospray interface (Applied Biosystems). Eluent A was 10 mM ammonium acetate in water and eluent B was acetonitrile. The flow rate was 200 $\mu\text{L}/\text{min}$ and a gradient was set as follows: 99% A for 2 min, then switched to 92% A, followed by a linear gradient to 30% A in 10 min, held at 30% A for 3 min, and back to the initial situation. The column was equilibrated with 99% A for 4 min. The electrospray voltage was set at 5,000 V, and the entrance and declustering voltages were set at 10 and 74 V, respectively. Tandem MS analysis was performed in positive multiple reaction monitoring mode between 2 and 12 min after injection. The liquid chromatography tandem MS instrument was controlled by Analyst software (version 1.4.2, Applied Biosystems). Standards for quantification were prepared by spiking blank material. These samples were processed together at the same time with real study samples.

Limit of detection of this method was 0.1 $\mu\text{g}/\text{mL}$ of ceftiofur for serum and lochia and 0.1 $\mu\text{g}/\text{g}$ of ceftiofur for endometrial tissue, respectively, as described in former studies (Drillich et al., 2006b).

Statistical Analyses

All data were recorded on data capture forms and transferred into a spreadsheet (Excel 2003, Microsoft, Munich, Germany). Descriptive statistical analyses were carried out with the SPSS statistic package (SPSS for Windows 16.0, SPSS Inc., Munich, Germany). Results are presented as means \pm standard error of mean. Level of significance was set at a P -value < 0.05 .

Correlations between concentrations of DCA in serum, endometrial tissue, and lochia were tested using the Spearman rank correlation (r_s) for nonparametric variables.

Table 1. Concentrations of desfuroylceftiofuracetamide (DCA; mean \pm SEM)¹ in serum, endometrial tissue, and lochia before (0) and 2 to 168 h after administration of ceftiofur crystalline free acid (CCFA; 6.6 mg/kg of BW)

Time after administration of CCFA (h)	n	Concentrations of DCA		
		Serum ($\mu\text{g/mL}$)	Endometrial tissue ($\mu\text{g/g}$)	Lochia ($\mu\text{g/mL}$)
0	6	0.10**	0.20*	0.10**
2	6	1.11 \pm 0.70	1.18 \pm 1.16	9.79 \pm 17.42
24	6	4.15 \pm 1.63	2.33 \pm 1.63	3.53 \pm 4.46
48	6	3.38 \pm 0.78	1.97 \pm 0.47	5.88 \pm 7.30
72	6	2.50 \pm 0.98	1.49 \pm 0.67	2.15 \pm 2.57
96	6	1.54 \pm 0.68	1.19 \pm 0.47	2.61 \pm 3.89
120	6	1.21 \pm 0.61	0.86 \pm 0.61	0.96 \pm 1.15
144	6	0.82 \pm 0.42	0.70 \pm 0.68	2.44 \pm 3.46
168	4	0.55 \pm 0.29	0.58 \pm 0.10	0.28 \pm 0.29*

¹MIC₉₀ for *Escherichia coli* <0.5 $\mu\text{g/mL}$; MIC₉₀ for *Arcanobacterium pyogenes*, *Fusobacterium necrophorum*, and *Prevotella melaninogenica* <0.125 $\mu\text{g/mL}$.

*Values below the MIC for *E. coli*; **values below the MIC for *A. pyogenes*, *F. necrophorum*, and *P. melaninogenica*.

RESULTS

Average gestation length of 5 cows was 280 ± 6 d. The remaining cow calved 3 wk before the calculated date of calving. Parturition occurred normally without intervention in all cows. All cows except one expelled the fetal membranes within 24 h after calving. Only one animal had a case of RFM. In all animals, samples were collected until 6 d after treatment. In 4 animals, additional samples were collected at 7 d after treatment.

Mean rectal temperature and mean concentration of BHBA of all cows for the whole observation period was $38.8 \pm 0.2^\circ\text{C}$ and 0.9 ± 0.4 mmol/L, respectively. Only 5 and 6 measurements exceeded 39.4°C and 1.4 mmol/L of BHBA, respectively.

The greatest DCA concentrations in serum were detected at 24 and 48 h after administration of CCFA in 50% of the cows (Table 1). Serum concentrations remained above the reported MIC of $0.5 \mu\text{g/mL}$ for *E. coli* during the entire observation period (Figure 1). In a few serum samples concentrations of DCA decreased below $0.5 \mu\text{g/mL}$ at 5 d ($n = 1$), 6 d ($n = 1$), and 7 d ($n = 2$) after administration of CCFA (Table 2). The concentrations of DCA in serum, however, remained above the MIC for *A. pyogenes*, *F. necrophorum*, and *P. melaninogenica* ($0.125 \mu\text{g/mL}$) for the entire observation period in all cows.

In endometrial tissue, mean concentrations of DCA remained above the MIC for *E. coli*, *A. pyogenes*, *F. necrophorum*, and *P. melaninogenica* during the entire observation period (Table 1; Figure 1). In 1 cow, however, concentrations of DCA decreased below the reported MIC for *A. pyogenes*, *F. necrophorum*, and *P. melaninogenica* at 6 d and in 2 cows below the reported MIC for *E. coli*, respectively, 5 and 6 d after administra-

tion of CCFA (Table 2). Mean concentration of DCA in endometrial tissue peaked 24 h after administration of CCFA and decreased until 7 d after treatment.

In lochia, DCA concentrations varied more widely. Mean concentrations of DCA in lochia exceeded the threshold values of $0.125 \mu\text{g/mL}$ (*A. pyogenes*, *F. necrophorum*, and *P. melaninogenica*) and $0.5 \mu\text{g/mL}$ (*E. coli*), respectively, until 6 d after administration of CCFA (Table 1; Figure 1). Mean concentrations were greatest 2 h after treatment. In individual cows, threshold values were not reached at certain time points (Table 2).

A highly significant positive correlation was found between concentrations of DCA in serum and endometrial tissue ($r_s = 0.979$; $P < 0.001$). The correlations between serum and lochia, and between endometrial tissue and lochia were considerably lower ($r_s = 0.32$ and $r_s = 0.48$, respectively, $P > 0.05$).

Concentrations of DCA measured in one cow with a case of RFM did not show differences compared with those in cows that expelled their fetal membranes within 12 h after calving until 5 d after administration of CCFA. Six days after administration of CCFA, however, concentrations of DCA in this cow decreased below the reported MIC for relevant pathogens (Table 2). Rectal temperatures of this cow increased on d 3 and 4 (40.0 and 39.6°C), respectively.

DISCUSSION

Treatment of puerperal uterine infections with systemic administration of antibiotics represents a widely accepted intervention in dairy cows (Brown et al., 2000; Okker et al., 2002; Drillich et al., 2006a). Ceftiofur has been reported to be the most commonly used antibiotic

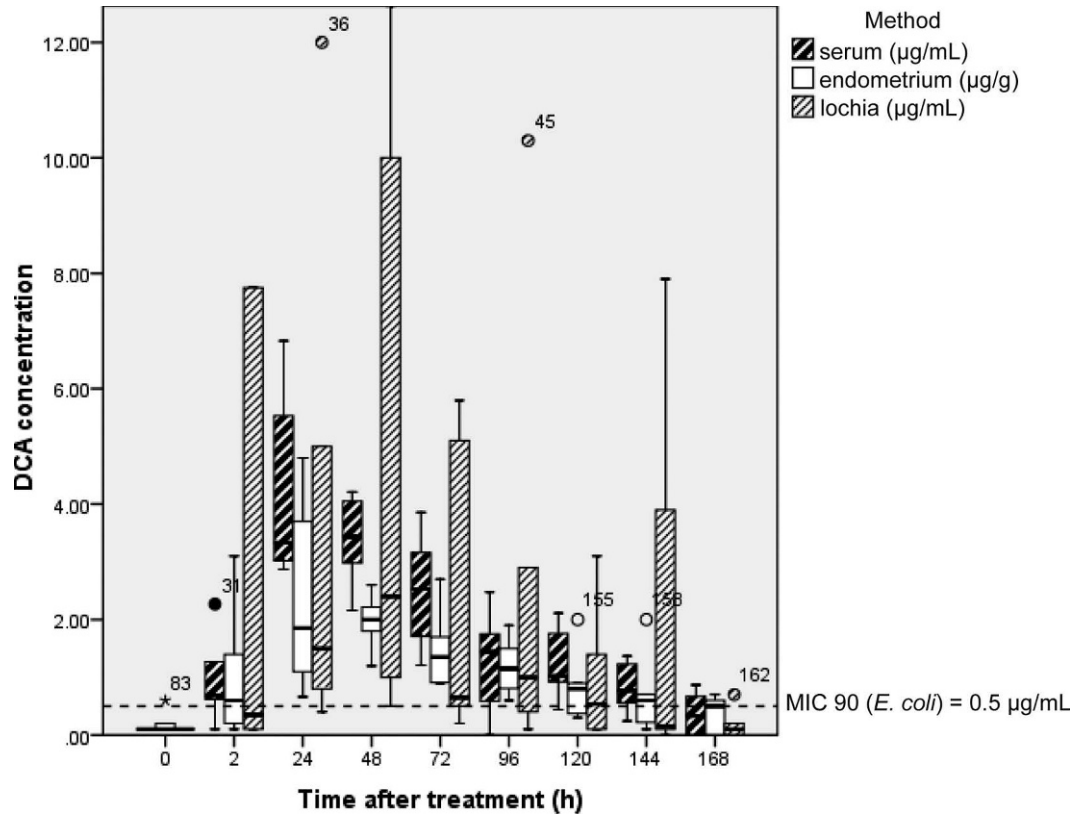


Figure 1. Concentrations of desfuroylceftiofuracetamide (DCA) in serum, endometrial tissue, and lochia before (0) and 2 to 168 h after administration of ceftiofur crystalline free acid (6.6 mg/kg of BW). Asterisk denotes an outlier before treatment.

drug in bovine practice (Zwald et al., 2004; Sawant et al., 2005). In the United States and Europe ceftiofur has been approved for the treatment of cows with acute postpartum metritis, bovine respiratory disease, and bovine interdigital necrobacillosis. Label instructions require treatment on 5 consecutive days. A survey on antibiotic usage conducted on 113 dairy farms identified failure to complete antimicrobial treatment course in 76% of the responding herds (Sawant et al., 2005).

According to anecdotal evidence the administration of systemic antibiotic drugs is often reduced to 2 to 3 d for practical or economic reasons. In addition to the abbreviated use of antimicrobials, the reliability on personal experience for antibiotic use and dosage could lead to inappropriate antibiotic usage (Sawant et al., 2005). Although a change to another antibiotic drug in case of resistance is not possible, a long-acting antibiotic drug approved for the treatment of uterine diseases in

Table 2. Number of samples with concentrations of desfuroylceftiofuracetamide (DCA) <0.5 and <0.125 µg/mL (serum and lochia)¹ or <0.5 and <0.125 µg/g (endometrial tissue)¹ after administration of ceftiofur crystalline free acid (CCFA; 6.6 mg/kg of BW)

Sample	Concentration of DCA	Time after administration of CCFA (h)							
		2	24	48	72	96	120	144	168
Serum	<0.5	—	—	—	—	—	1/6 ²	1/6 ²	2/4 ²
	<0.125	—	—	—	—	—	—	—	—
Endometrium	<0.5	1/5	—	—	—	—	2/6 ²	2/6 ²	—
	<0.125	1/5	—	—	—	—	—	1/6 ²	—
Lochia	<0.5	2/5	1/6	1/6	—	2/6	3/6	3/5 ²	3/4 ²
	<0.125	2/5	1/6	—	—	1/6	2/6	2/5 ²	2/4 ²

¹MIC₉₀ for *Escherichia coli* <0.5 µg/mL; MIC₉₀ for *Arcanobacterium pyogenes*, *Fusobacterium necrophorum*, and *Prevotella melaninogenica* <0.125 µg/mL.

²One sample from one cow with retained fetal membranes included.

cows would be advantageous from a practical, economic (Galligan, 2006), and prudent drug use perspective. The objective of the present study was to determine concentrations of ceftiofur derivatives in serum, endometrial tissue, and lochia after one s.c. administration of CCFA, a sustained-release formulation of ceftiofur, in healthy puerperal dairy cows.

Time above the MIC is the pharmacokinetic (PK) parameter most associated with efficacy for this class of antibiotics. Overall, mean concentration of DCA in serum, uterine tissue, and lochia remained above the reported MIC for common uterine pathogens of 0.5 µg/mL for *E. coli* (Sheldon et al., 2004) during the entire study period of 7 d. In single samples, however, 6 and 7 d after administration of CCFA, concentrations of DCA decreased to <0.5 µg/mL (1, 2, 3 and 2, 0, 3 samples in serum, endometrial tissue, and lochia on d 6 and 7, respectively; Table 2). Concentrations of DCA in serum remained above the MIC for *A. pyogenes*, *F. necrophorum*, and *P. melaninogenica* during the entire observation period. Concentrations of DCA in endometrial tissue decreased below 0.125 µg/mL in only one cow at d 6. Thus, only in few instances, suboptimal antibiotic concentrations occurred. As it is known that true tissue concentrations, especially of water-soluble antibiotics, are underestimated due to dilution in the extracellular fluid during the analytical process (Brown et al., 1995), in vivo and in vitro efficacy of antibiotics may differ. Thus, true antibiotic concentrations also may be underestimated in the present study.

Pharmacokinetic data of ceftiofur hydrochloride have been described after s.c. and i.m. administration in healthy dairy cows (Brown et al., 2000; Okker et al., 2002). Several studies demonstrated that active metabolites of ceftiofur hydrochloride exceeded the reported MIC of uterine pathogens for 24 h in cows (Brown et al., 2000; Okker et al., 2002; Drillich et al., 2006b) and mares (Witte et al., 2010). Additionally, a recent study demonstrated the efficacy of 3 consecutive administrations of ceftiofur hydrochloride in cows with RFM (Drillich et al., 2006a).

A single administration of CCFA has been demonstrated to be clinically efficacious for treatment of acute bovine interdigital necrobacillosis and to achieve serum DCA concentrations above the MIC of relevant pathogens for 6 d (Van Donkersgoed et al., 2008). The present study is the first providing PK data on concentrations of active metabolites in lochia and uterine tissue after administration of CCFA at a single dose of 6.6 mg/kg of BW.

Pharmacokinetic data of CCFA were different compared with those described for ceftiofur hydrochloride. Okker et al. (2002) and Drillich et al. (2006b) found a first maximum of ceftiofur derivatives in serum and

endometrial tissue 2 h after treatment, whereas in our study, greatest DCA concentrations were reached 24 h after administration. This can be explained by the slow release characteristic of the CCFA formulation. Additionally, maximum values found in the present study in serum (4.15 ± 1.63 µg/mL) and endometrial tissue (2.33 ± 1.63 µg/g) were greater than the maximum values reported by Okker et al. (2002; 2.85 µg/mL and 2.23 µg/g) and Drillich et al. (2006b; 1.66 µg/mL and 2.02 µg/g).

For lochia, results were more heterogeneous. This is in agreement with previous studies (Okker et al., 2002; Drillich et al., 2006b) as well. The lower concentrations of DCA in lochia compared with those in serum and endometrial tissue may occur due to a dilution of lochia with the remaining fluid within the uterus after parturition (Okker et al., 2002). Mean concentrations of DCA in lochia, however, decreased below the MIC for *E. coli* (0.5 µg/mL) only 7 d after administration and stayed above the MIC for *A. pyogenes*, *F. necrophorum*, and *P. melaninogenica* (0.125 µg/mL) during the whole observation period (Table 1). Thus, concentrations of DCA measured in lochia in the present study are likely to be effective against relevant pathogens. The large variations of DCA concentrations in lochia contributed to the moderate correlations of concentrations in lochia compared with serum and endometrial tissue.

In the present study, relationships between rectal temperature and ceftiofur derivatives in serum, endometrial tissue, and lochia were not detected as has been described in former studies (Drillich et al., 2006b). As rectal temperature was measured every 24 h immediately before, but not after, sampling, the effect of intrauterine manipulation due to sampling could not be demonstrated. An increase of rectal temperature as shown by other authors 2 and 4 h after sampling (from 38.8°C to 39.2°C; Drillich et al., 2006b) could not be shown in the present study. Therefore, no evidence exists that temperature increases due to the sampling procedure. Another explanation could be that an increased ceftiofur concentration leads to a release of pyretic toxins due to the increased bactericidal potential of the antimicrobial drug (Drillich et al., 2006b). In the present study, however, rectal temperatures were lowest at the time of greatest DCA concentrations in serum and endometrial tissue.

This study provides the first evidence that concentrations of ceftiofur derivatives in serum, endometrial tissue, and lochia of healthy puerperal dairy cows remained above the MIC of common uterine pathogens over a 7-d observation period after s.c. administration of 6.6 mg/kg of BW CCFA. Only in single samples, 6 and 7 d after administration of CCFA, concentrations decreased below the reported MIC of relevant patho-

gens, which would temporarily lead to suboptimal drug concentrations. Furthermore, concentrations of DCA decreased below the MIC already 5 d after administration of CCFA in one cow with RFM. Further research is warranted to investigate PK data after one administration of CCFA in cows with postpartum uterine diseases (e.g., RFM, APM).

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