Pharmacokinetics of ceftiofur sodium in pregnant pony mares

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Abstract

Placentitis is a common cause of pregnancy loss in the mare. Effective treatments for placentitis remain elusive. Ceftiofur sodium is effective against the most common pathogens causing placentitis, including *Streptococcus equi* subspecies *zooepidemicus*. However, little is known about the pharmacokinetics of the drug in pregnant mares or whether or not the drug penetrates the fetal placental barrier. Eight pregnant pony mares were treated with ceftiofur sodium (Naxcel®)(4.4mg/kg, IM, q24h) for at least 4 days prior to induction of parturition. Mares were monitored daily for signs of onset of parturition and mammary secretions were tested daily for calcium concentrations using a commercial test. Parturition was induced using oxytocin (5 IU) administered at 25 minute intervals until the onset of parturition. Allantoic fluid, amniotic fluid and pre-suckle colostrum samples were collected. Plasma samples were collected at 2 hours post drug administration in the mare, and at birth (time point 0) and at 1,2,4,8, and 24 hours after delivery from mares and foals. Plasma samples were analyzed using high performance liquid chromatography to detect concentrations of ceftiofur and its active metabolite, desfuroylceftiofur acetamide (DCA). Median plasma concentrations of DCA of 8.61 (7.38-8.89) µg/ml were found at birth in the mare, while no detectable concentrations of the drug were found in foal plasma. Median DCA concentrations of 1.70 (1.30-1.75) µg/ml were found in milk. No detectable amounts of the drug were found in either the allantoic or amniotic fluid. Results from this study show that therapeutic concentrations of ceftiofur sodium seem not to attain the fetal compartment or foal plasma after drug administration to the mare prior to delivery. These data suggest that ceftiofur sodium does not penetrate the equine feto-placental barrier and, therefore, may not be effective for treating mares with placentitis.

Introduction

Equine placentitis is a common cause of abortion or stillbirth in horses, and is primarily caused by ascending vaginal infections with *Streptococcus equi zooepidemicus, Klebsiella pneumoniae, Leptopspira, Escheria coli*, other streptococcal species or fungi(1). The bacterial infection is established in placental tissue and triggers a secondary inflammatory reaction involving the release of pro-inflammatory cytokines from the chorioallantois membrane. The release of these cytokines in turn stimulates the release of PGE2 and $PGF2_{\alpha}$ into the allantoic fluid causing premature udder development, vulvar discharge and, eventually, premature delivery (2, 3). A 24-year retrospective study (n=1822) found that 64% of diagnosed, premature deliveries were caused by placental infections (4). This statistic demonstrates the need for a reliable treatment protocol, but to date the efficacy of treatment strategies has been inconsistent.

Treatment protocols currently used in equine practice are directed to different aspects of the disease. Antimicrobials commonly used in practice include potassium penicillin G, trimethoprim sulfamethoxazole (TMPS) and gentamicin. Following application to late gestation mares, these antibiotics have been detected in allantoic fluid using *in vivo* micro-dialysis at concentrations exceeding the minimum inhibitory concentration (5,6) Treatments combining an antibiotic and an anti-inflammatory agent, such as pentoxyfiline, has shown to prolong gestation, but not to reliably prevent pre-term delivery or fetal death(7). By contrast, a study conducted to

determine the efficacy of a combination of TMPS, pentoxifyline and altrenogest suggested that this combination improves fetal viability, although the TMPS was not able to eliminate the bacteria from the uterus (7). Although commonly used in practice, the inconsistent efficacy of TMPS against *S. zooepidemicus* is a major disadvantage for this drug as a first-line treatment for placentitis and suggests the need for further studies into antimicrobials effective *in vivo* against *S. equi zooepidemicus* and other agents commonly associated with placentitis.

Ceftiofur sodium, a third generation cephalosporin (8), has been shown to be highly active against *S. equi zooepidemicus* and has a broad spectrum of activity covering many Gram-positive aerobes and some anaerobes (8, 9). The high sensitivity of *S. equi zooepidemicus* to ceftiofur is endorsed by studies on *in vivo* bronchopneumonia infections with *S. equi zooepidemicus* (10). Ceftiofur has been shown to penetrate various body tissues including the lung, endometrium (although in low concentrations) and joints (11, 12). A study in cattle detected ceftiofur sodium in the caruncles of retained fetal membranes (13). Although all of this work seems to provide evidence for its activity, recent work from the University of Florida indicated a poor penetration of the fetal placental unit by the long-acting preparation of ceftiofur; ceftiofur crystalline free acid (CCFA) (14). The objective of this study was to determine the pharmacokinetics of ceftiofur sodium in pregnant pony mares and to evaluate the distribution of the drug into the fetal placental tissues.

Materials and Methods

Administration of ceftiofur sodium

Seven healthy pregnant mixed breed pony mares of various ages were enrolled in the study at day 300 of gestation. The average weight of the mares at that time was 403,29 kg (± 47,02 kg). All mares were monitored closely for impending parturition on the basis of changes in mammary gland development, mammary secretion calcium concentrations and vulvar softening. Mammary secretions were tested for calcium carbonate concentrations using an on-site test (FoalWatch™, Chemetrics, Calverton, VA). When noticeable changes in mammary gland development were observed, mares were administered ceftiofur sodium (Naxcel®, Pfizer Animal Health, Kalamazoo, MI, USA, 4.4 mg/kg, IM, q24h) for at least four days prior to induction of parturition, at the same time in the morning of each day, according to the manufacturer's instructions. Mammary secretion calcium carbonate concentrations were tested daily, in the evening, to detect a concentration of ≥ 200 ppm. Once this calcium carbonate concentration had been attained, induction of parturition was planned.

Induction of parturition

Parturition was induced approximately 1.5 hours after the administration of the last dose of ceftiofur sodium to best monitor sample drug concentrations after administration. The mare was brought to a small paddock or large deep-bedded stall. The perineal area was washed and the tail was wrapped. A vaginal examination was performed immediately prior to administration of oxytocin, to examine cervical width and softness. Oxytocin (5 IU IM per animal) was administered, and mares were closely monitored for rupture of the chorioallantois. Subsequent doses of oxytocin were administered every 25 minutes (5IU IV) preceded by a vaginal examination until the onset of parturition. Mares were allowed to foal naturally unless dystocia or premature placental separation occurred.

Sampling

Following rupture of the chorioallantois membrane, allantoic fluid was collected by free-catch and stored. Amniotic fluid was collected by puncture of the amnion using a 16G needle and a 60ml syringe. Blood samples were collected for plasma (10ml EDTA tube) from mares at two hours post-administration of the ceftiofur sodium. Blood samples for plasma were obtained from mares and foals at birth (T=0), and at T = 1, 2, 4, 8 and 24 hours post-partum . The blood samples were centrifuged (400 g, 10 minutes) and stored at -80°C until analysis. Pre-suckle colostrum was collected from the mare at the time of birth.

HPLC analysis

HPLC was used for ceftiofur sodium detection because a previous study had reported its use for this purpose (15). Ceftiofur was extracted from plasma, colostrum, allantoic and amniotic fluid as the desfuroylceftiofur-like metabolites using a dithioerythritol solution and captured in a C-18 solid phase extraction cartridge (Varian Inc, Walnut Creek, Ca, USA). Desfuroylceftiofur metabolites were converted to desfuorylceftiofur acetamide (DCA) using iodacetamide. An isocratic mobile phase with a pH of 4 (7% acetonitrile, 1% acetic acid_ to which 90 mg/L heptane sulphonic acid was added) and a C18 (4µm, 3.9x150 mm) column was used to carry out the analysis.
For quality control, a reference standard of ceftiofur (Sigma-Aldrich, St. Louis, MO, USA) was used to produce a standard curve (R^2=0,997). Standard solutions of 0.5, 1, 3, 5, 7, 10, 12, 15 and 20 µg/ml of ceftiofur were run with each sample. The limit of detection was 0.1 µg/ml.

Statistical Analysis

Data were evaluated for the normality using Shapiro-Wilks test and equality of variances using Levenes test (SPSS 20, IBM). After proving normality and equality of variances, pharmacokinetic parameters were calculated using a non-compartmental analysis. **Results** Desfuorylceftiofur acetamide (DCA) concentrations in blood plasma samples and colostrum are shown in Table 1.

Table 1: Median detected DCA concentrations (lower and upper quartile) in mare blood plasma at different time intervals. Time intervals represent hours after birth of the foal.

Sample type	**DCA concentration (µg/ml)**
2 hrs post administration	8.61 (7.38-8.89)
0 (birth)	8.08 (7.76-8.38)
1	7.20 (6.12-7.83)
2	6.25 (5.29-6.77)
4	4.15 (3.46-4.58)
8	2.21 (1.95-2.36)
24	0.20 (0.14-0.35)
Colostrum	1.70 (1.30-1.75)

Trace amounts of DCA were detected in the plasma of 2 foals, 8 hours after birth. The amounts found were below the detection limit of <0.1 µg/ml and therefore not quantified.

Table 2 displays the concentrations of DCA detected in allantoic and amniotic fluid.

Table 2: DCA concentrations in allantoic and amniotic fluid for the 7 mares enrolled in the study.

Mares	**Allantoic Fluid**	**Amniotic Fluid**
1	<0.1 µg/ml	ND
2	<0.1 µg/ml	ND
3	ND	ND
4	ND	ND
5	<0.1 µg/ml	ND
6	<0.1 µg/ml	ND
7	7,2 µg/ml	ND

Pharmacokinetic parameters as calculated using a non-compartmental analysis are summarized in table 3.

Table 3: Pharmacokinetic parameters displayed as median (lower- upper quartile) for the 7 mares enrolled in the study.

Pharmacokinetic parameter	
C_{max} (µg/mL)	8.61 (6.62-10.05)
C_{min} (µg/mL)	2.21 (1.26-2.72)
C_{avg} (µg/mL)	3.07 (2.19-3.44)
AUC (µg·h/mL)	74.85 (47.30-84.21)
β (h^{-1})	0.14 (0.10-0.22)
$t_{1/2\beta}$ (h)	4.78 (3.20-6.84)
MRT (h)	7.49 (5.52-9.93)
Accumulation index	1.03 (1.01-1.10)

Discussion

The aim of this study was to evaluate the distribution of ceftiofur sodium into the fetus and placental membranes during late gestation in mares. Therapeutic concentrations of ceftiofur sodium, in the form of the desfuroylceftiofur metabolites, were not detected in fetal fluids or foal blood plasma following repeated daily administration of the antibiotic to the mare. The peaks shown by HPLC were below the detection limit and probably represent false positive due to a technical error. The 7.2 µg/ml found in the seventh mare's allantoic fluid was most likely caused by contamination of the allantoic fluid with urine, during free-catch. By contrast, expected concentrations of DCA metabolites were detected in mare blood plasma (Table 1), and low levels of the drug were detected in the colostrum. Pharmacokinetic parameters (Table 3) were comparable to previously reported data (15, 18, 19). The maximum plasma drug concentrations found in previous studies vary (15, 18, 19), but the values detected in our study are well above the values recorded in the earlier studies and those reported in dose determination studies by Pfizer Animal Health (20). The maximum plasma concentrations (C_{max}) found in this study were significantly higher than in previous studies ($P< 0,05$). This was expected, because a dose of 4.4 mg/kg was administered in this study, while in previous studies only 2.2 mg/kg dose administered in These data suggest that ceftiofur sodium does not penetrate the equine fetal placental unit and, therefore, would not be effective for treating mares with placentitis.

The cause of this poor penetration of cetiofur across the placenta remains unknown. However, the equine placenta is an epitheliochorial placenta designed to only selectively allow the penetration of xenobiotics, hence protecting the foal against undesirable exposure to toxic plant metabolites. Three different mechanisms contribute to the passage of molecules across this barrier; simple diffusion, facilitated diffusion and phago- and pinocytosis (16) . Most drugs cross the placenta by simple, gradient supported diffusion. Substance specific factors affecting the rate at which a drug crosses the placental membranes include; drug concentration in maternal blood molecular weight of the drug, lipid solubility, degree of protein binding and degree of ionization/polarization of the molecule at physiological tissue pH conditions (17).

The epitheliochorial placenta of the mare contains diffusion pores through which substances can diffuse towards the fetus. These pores are a limiting factor for molecules with a higher molecular weight. Research has shown that molecules > 500 D are unlikely to completely pass the placenta in man. In the sheep, even molecules of 180 D have found to be unable to pass across the placenta (16). While sheep have a different type of epitheliochorial placenta to horses, there are also various similarities. It is therefore possible that ceftiofur with a molecular weight of 523 D(21) and which is highly bound to proteins is simply too large to cross the placenta of the horse by simple diffusion.

The third factor; lipid solulibility can be estimated using the octanol:water partition coefficient (log K_{ow}). This value measures how lipophilic a drug is, and thus its likely affinity for the lipid bilayer of the cell membrane. The log K_{ow} of ceftiofur has been reported to be 1.6 suggesting that it is a lipophobic substance(21, 22). This suggests that the poor transfer of ceftiofur sodium across the equine fetal-placental unit may be, at least partly, explained by low uptake of the substance by cell membranes.

When metabolized to desfuroylceftiofur, ceftiofur reversibly binds to plasma and tissue proteins (15). Plasma protein binding of drugs has been found to be correlated directly with the distribution of drugs over the placenta (23)and could therefore affect the ability of ceftiofur to enter the fetus, as found in the present study.

The last of the substance specific factors is the rate of ionization and polarization at physiological pH. The extent to which ceftiofur is ionized, is reflected by its' pKa value. The pKa value of ceftiofur sodium is estimated at 3.7, suggesting that ceftiofur sodium in the bloodstream (pH 7.4) would act as a weak acid. Ionized molecules are less likely to cross over the fetal-placental barrier, again inhibiting distribution of ceftiofur into placenta and foal.

An additional factor influencing concentrations found in fetal tissues and fluids is the clearance of the drug by the fetus. Although important in studies evaluating fetal drug exposure, in our study fetal clearance seems an irrelevant factor as there is apparently no transport across the placenta.(11)

Previous studies used different methods to determine the pharmacokinetics of ceftiofur in horses. Both bio-assays and HPLC have been described. HPLC was studied as a procedure with high sensitivity and specificity, and therefore suitable for pharmacokinetic studies on third generation cephalosporins (24). The quality and precision of HPLC analysis is supported by the low relative standard deviation (%RSD) values found during the procedure. The precision of this study as a whole is limited due to the small sample size (n=7). Although it is a small sample, the results look pretty consistent and therefor suggest that a bigger sample would show the same results.

Another shortcoming would be the variation of days the mares were treated. This variation was caused by the difficulty on predicting the right time for the induction of parturition. The effect of this variation can be considered insignificant because of the short half-life value of ceftiofur sodium.

In conclusion, results of this study fail to provide evidence that cetiofur crosses the placenta in pre-partum horses. Although on the basis of the results of this study, ceftiofur sodium could not be recommended as part of a treatment protocol for placentitis in mares, it cannot be entirely excluded that endometrial tissue levels would reach concentrations that are therapeutically effective. More studies on the physiology of the equine placenta are necessary to predict which drug(s) would be better alternatives for treatment of mares with clinical placentitis. Research on packaging of ceftiofur in liposomes in cows has shown promising results in the transfer of ceftiofur towards the udder (25) and might be useful as a future transporter for drugs across the placenta.

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