A Microdialysis Model to Detect Drugs in the Allantoic Fluid of Pregnant Pony Mares (21-Nov-2003)

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Abstract
Placental transfer of penicillin and gentamicin occurs both in normal pregnant mares and mares with placentitis. Microdialysis is a useful technique for monitoring therapeutic drug levels in the equine allantoic fluid and should aid development of effective treatment protocols for mares with high-risk pregnancies.

1. Introduction
Ascending placentitis is a common cause of equine abortion. Current treatment protocols exhibit limited success against premature parturition. It is conceivable that therapeutic agents are ineffective because of inadequate drug levels within target tissues. Little is known about the transfer of drugs across the equine placenta, and studies to date have been unable to consistently detect drug concentrations in either the allantoic fluid of pregnant mares or in fetal tissue [1,2]. The lack of drug transfer noted in these studies may be related to insensitive sampling techniques. Previous studies have used repeated transabdominal allantocentesis as a means of obtaining samples for pharmacokinetic analysis. Using this technique, allantoic fluid samples must be purified before analysis, and the alteration of the sample may reduce the sensitivity of the technique. Furthermore, complications such as ascending bacterial infection and abortion have been associated with repeated allantocentesis in mares late in pregnancy [3]. A novel technique, using in vivo microdialysis, provides a highly sensitive method of detecting drugs in blood, body fluids, and tissue extracellular fluids. This technique affords several advantages over other sampling techniques, including cleaner samples (devoid of protein and cells), reduced disruption of delicate blood-tissue barriers, [4] and continuous measurement of non-protein-bound drug over time. Furthermore, there is no net removal of body fluid during sampling, because each small aliquot (<20 µl) is replaced with an equal volume of saline solution through the microdialysis system. Recently, in vivo microdialysis probes have been used successfully in horses to evaluate the pharmacokinetics of drugs in skeletal muscle and in the central nervous system (CNS) [5]. The primary objective of this study was to validate the use of an in vivo microdialysis system for monitoring placental transfer of selected drugs into the allantoic cavity of pregnant pony mares. We hypothesized that microdialysis would be a sensitive technique for measurement of penicillin G potassium, gentamicin, and flunixin meglumine within the allantoic cavity of pregnant mares.

2. Materials and Methods
Five normal pregnant pony mares (ages 4 - 15 yr) were enrolled sequentially at 269 - 271 days of gestation. Baseline inclusion data (normal systemic parameters: combined thickness of the utero-placental unit <8 mm [6]; fetal fluid character, fetal activity, and heart rate within normal limits [7]) were collected before experimentation.
Mares were restrained in stocks and sedated using detomidine hydrochloride [a] (20 µg/kg, IV) and butorphanol [b] (20 µg/kg, IV). A 16-gauge catheter was placed in the left jugular vein for collection of peripheral blood samples. The mare's abdomen was scanned using a 3.5-MHz sector ultrasound probe [c] to identify a significant allantoic fluid pocket. The abdominal area adjacent to the fluid pocket was clipped and aseptically prepared with Betadine® scrub and alcohol. Using ultrasound guidance, a 17-gauge spinal needle [d], preloaded with a 0.25-mm diameter microdialysis probe [e] with a molecular weight (MW) cut off of 5 KdA MW, was inserted into the allantoic cavity. The correct position of the
A microdialysis probe was verified with allantoic fluid analysis. A second microdialysis probe was placed in the right jugular vein, using a 16-gauge catheter as a guide. The jugular vein microdialysis probe was used to validate the efficiency of the microdialysis system for detecting drugs, and this was achieved using analysis for penicillin G concentrations. Penicillin V was perfused continuously through the probe to measure probe function and efficiency throughout the experimental period. Penicillin G potassium (22,000 IU/kg, IV, q 6 h), gentamicin (6.6 mg/kg, IV, q 24 h), and flunixin meglumine (1 mg/kg, IV, q 24 h) were administered to the mares. Microdialysis and serum samples were collected at set time intervals over a 24-h period and stored at -80°C.

Ten days after the initial experiment, placentitis was induced in two mares to generate preliminary information regarding placental drug transfer in infected mares. Ascending placentitis was induced through intra-cervical inoculation with Streptococcus equi zooepidemicus (10⁷ CFU). Briefly, the inoculum was placed 2 cm into the external ostium of the cervix using a sterile artificial insemination (AI) pipette. Minimum inhibitory concentrations (MIC) for both antibiotics were determined prior to experimentation based on susceptibility results for the above bacterium. Mares were fitted with microdialysis probes as described above. Microdialysis samples were obtained from infected mares continuously for 24 h and stored at 80°C until analysis.

Drug levels were measured in all samples using high performance liquid chromatography with ultraviolet (UV) detection (penicillin and flunixin) or enzyme-linked immunosorbent assay (ELISA) (gentamicin). Descriptive data analysis was performed and tested for normality, MIC levels were indicated, and all results were reported as means with associated SE.

### 3. Results

#### Peripheral Venous Drug Concentrations

**Serum Samples** - Peak serum concentrations (mean ± SE) of penicillin G for the five normal mares were 53.65 ± 37.12 µg/ml at 5 min post-drug injection, and levels remained detectable beyond 120 min post-injection (0.62 ± 0.53 µg/ml). Gentamicin serum concentrations for the same group of mares were 47.07 ± 17.15 µg/ml at 15 min post-drug injection, and levels remained detectable beyond 1,080 min post-injection (0.10 ± 0.04 µg/ml). Flunixin meglumine serum concentrations were 19.83 ± 6.36 µg/ml at 5 min post-drug injection, and levels remained detectable beyond 1,440 min post-injection (1.44 ± 0.27 µg/ml).

**Microdialysis Jugular Samples** - Microdialysis drug concentrations for penicillin G (sampled from the right jugular vein) showed the same temporal pattern as serum concentrations. Analyses of gentamicin and flunixin meglumine concentrations were not performed, because penicillin data was sufficient for validation purposes. Penicillin V concentrations from the jugular microdialysis probes demonstrated effective probe function for the duration of the experiment in all mares. This internal standard (penicillin V) was the only validation method used for the infected mares.

#### Allantoic Drug Concentrations

**Normal Mares** - Peak allantoic concentrations (mean ± SE) of penicillin G for the five normal mares were 9.78 ± 4.81 µg/ml at 45 min post-drug injection, and levels remained detectable beyond 210 min post-injection (0.93 ± 0.91 µg/ml). MIC (approximately 0.03 µg/ml) for penicillin G were detectable 15 min post-drug injection (5.98 ± 2.88 µg/ml) and remained above MIC levels for a period of 210 min post-injection (0.93 ± 0.91 µg/ml).

Peak gentamicin allantoic concentrations were 8.49 ± 6.86 µg/ml at 105 min post-drug injection, and levels remained detectable beyond 1,260 min post-injection (0.05 ± 0.04 µg/ml). MIC levels (approximately 4 µg/ml) for gentamicin were detectable 45 min post-drug injection (4.9 ± 5.0 µg/ml) and remained detectable for a period of 330 min post-injection (4.4 ± 3.09 µg/ml).

Flunixin meglumine concentrations were undetectable in the allantoic microdialysates.

**Infected Mares** - Peak allantoic concentrations (mean) of penicillin G for the two infected mares were 11.23 µg/ml at 15 min post-drug injection, and levels remained detectable beyond 210 min post-injection (0.63 µg/ml). MIC levels for penicillin G were achieved within 15 min (11.23 µg/ml) and were detectable up to 210 min post-injection (0.63 µg/ml).

Peak gentamicin serum concentrations were 3.88 µg/ml at 75 min post-drug injection, and levels remained detectable beyond 1,260 min post-injection (0.0725 µg/ml).

Gentamicin MIC levels were only obtained in one of the two mares 75 min post-drug injection (5.37 µg/ml), and it remained detectable up to 150 min post-injection (4.64 µg/ml).

Penicillin G and gentamicin allantoic concentrations for infected mares showed a similar temporal pattern compared with allantoic concentrations obtained in the five normal mares.
4. Discussion
These data confirm that the microdialysis system is a useful technique for measuring concentrations of penicillin G and gentamicin in equine allantoic fluid of normal and infected pregnant pony mares. Concentrations of penicillin G and gentamicin were lower in the allantoic fluid but remained detectable for a longer period of time than serum concentrations, possibly reflecting a compartmentalization of the drugs.
Flunixin meglumine concentrations were not detected in microdialysates of allantoic fluid. However, because flunixin meglumine is highly bound (98%) to serum protein molecules (approximately 60 - 100 KDa MW), it would be unable to penetrate the microdialysis membrane.
Additionally, data from this study show that penicillin G penetrates the allantoic cavity and attains concentrations in allantoic fluid that are comparable with concentrations in the systemic circulation; additionally, allantoic drug concentrations remain at therapeutic levels for a period consistent with concentrations in blood. This refutes previous work [1] indicating that penicillin G is not able to penetrate the allantoic cavity in pregnant mares. Differences between these two studies may be attributed to the increased sensitivity of the microdialysis system for detecting drugs in allantoic fluid.
Concentrations of gentamicin in allantoic fluid were lower than serum concentrations. Furthermore, MIC did not meet the 8 to 10-fold increase over baseline MIC (4 µg/ml) necessary for inhibition of *Streptococcus equi zooepidemicus*. This suggests that penetration of gentamicin in the allantoic cavity would probably not be effective for this organism. However, allantoic gentamicin concentrations seem to be adequate for treatment of placental infections caused by *E. coli* and *Klebsiella spp* (MIC < 1 µg/ml).
Both penicillin and gentamicin were also detected in the allantoic fluid of mares with experimentally induced placentitis. Conclusions regarding temporal pharmacological patterns in normal versus infected mares cannot be made because of small sample numbers.
Few side effects were associated with the technique. The range of allantoic puncture attempts to place the microdialysis probe was 1 - 3 per session. Some mares (1 of 9) were involved in two probe placements on different days and up to three sticks without adverse side effects. One pony mare aborted after three allantoic puncture attempts on 1 day. Although few side effects were experienced using allantoic puncture in this study, as few as one allantoic stick induced abortion in previous work [i]. Therefore, although effective for sensitively monitoring drug concentrations in an experimental setting, the expense (approximately $1,700/experiment) and sensitive nature of the equipment and the potential risk to the pregnancy would preclude the use of this technique in a clinical setting.
In conclusion, this technique was effective for measuring penicillin G and gentamicin in allantoic fluid of pregnant pony mares. Studies are ongoing to determine the efficacy of several therapeutic agents with regards to penetration of fetal membranes. These studies should aid in the development of reliable treatment protocols for mares with placentitis.

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Footnotes
[a] Dormosedan, Pfizer, Orion, Division of Pfizer Inc., New York, NY 10017.
[b] Torbugesic, Fort Dodge, IA 50501. %c], Alok 900, Alok Co. LTD, Tokyo, Japan, 181-8622.
[d] EchoTip Disposable Lancet Needle 17-gauge 15 cm, Cook ObGyn, Spencer, IN, 47460.
[f] Penicillin G potassium, Pfizerpen, Roerig, Division of Pfizer Inc., NY, NY 10017.
[g] Gentocin, Schering-Plough, NJ, 07083.
[h] Banamine, Schering-Plough, NJ, 07083.

References
3. LeBlanc MM, Giguere S, Brauer K, et al. Premature delivery in ascending placentitis is associated with increased


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