The Veterinary Journal 202 (2014) 314–322

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Ultrafiltration of equine digital lamellar tissue

Claire Underwood *, Simon N. Collins, Andrew W. van Eps, Rachel E. Allavena, Carlos E. Medina-Torres, Christopher C. Pollitt

Australian Equine Laminitis Research Unit, School of Veterinary Science, University of Queensland, Gatton Campus, Gatton, Qld 4343, Australia

ARTICLE INFO

Article history:
Accepted 3 May 2014

Keywords:
Horse
Laminitis
Microdialysis
Ultrafiltration
Pharmacology

ABSTRACT

There are no experimentally validated pharmacological means of preventing laminitis; however, locally acting pharmaceutical agents with the potential to prevent laminitis have been identified. Demonstrating therapeutic drug concentrations in lamellar tissue is essential for evaluating the efficacy of these agents. The aim of this study was to develop an experimental technique for repeatedly sampling lamellar interstitial fluid. A technique for placing ultrafiltration probes was developed in vitro using 15 cadaver limbs. Subsequently, lamellar ultrafiltration probes were placed in one forelimb in six living horses. Interstitial fluid was collected continuously from the probes as ultrafiltrate for 4 ($n = 4$) or 14 days ($n = 2$). The rate of ultrafiltrate collection was calculated every 12 h. Biochemical analyses were performed on ultrafiltrate collected on night 1 (12–24 h post-implantation) and night 4 (84–96 h post-implantation). Sections surrounding the probe and control tissue from the contralateral limb were harvested, stained with H&E and Masson's trichrome and scored based on the tissue response to the probe.

Ultrafiltration probes were placed in the lamellar tissue in all six horses. Ultrafiltrate was collected from these probes at 55 (30–63) μL/h (median [interquartile range]). Fluid production decreased significantly with time from night 3 onwards ($P < 0.05$). There was no significant change in the constituents of the ultrafiltrate between nights 1 and 4 ($P > 0.05$). The technique was well tolerated. This study demonstrates that ultrafiltration can be used to sample equine digital lamellar interstitial fluid, and has potential for measuring lamellar drug levels.

Introduction

Acute laminitis is a common sequel to a variety of primary diseases in the horse (Parsons et al., 2007). Once laminitis occurs, mechanical failure of the lamellar tissue is unavoidable and so prophylaxis is key. Digital hypothermia prevents laminitis in the oligofructose model described by van Eps and Pollitt (2009a) but there are no experimentally validated pharmaceutical means of laminitis prophylaxis. Inflammation, proteolytic enzymatic activation and basement membrane degradation have been implicated in the pathophysiology of sepsis-related laminitis (Pollitt et al., 1998; Belknap et al., 2007; Visser, 2009) and pharmaceutical agents that may block these pathways at the lamellar level have been proposed (Pollitt et al., 1998; Leise et al., 2012; Wang et al., 2013). The first step in evaluating pharmacological laminitis prophylaxis is to establish a delivery mechanism that yields sustained therapeutic concentrations in the lamellar tissue. This requires a means of repeatedly measuring lamellar drug concentrations.

Measurement of drug concentrations within lamellar tissue is challenging. Techniques to determine tissue drug concentrations include implanted tissue cages, biopsies, microdialysis and ultrafiltration (Papich et al., 2002; Davis et al., 2006; Voermans et al., 2006; Nourian et al., 2010b). Tissue cages are difficult to implant because of the anatomical location of lamellar tissue beneath the hoof capsule. Serial lamellar biopsies (Visser and Pollitt, 2011) are invasive and the resultant inflammation (Visser, 2009) may affect drug distribution (Barza and Cuchural, 1985). Homogenised biopsy material may under- or over-estimate drug concentrations (Cars and Ogren, 1985; Nix et al., 1991). Lamellar microdialysis uses small, implantable probes that cause minimal tissue damage (Nourian et al., 2010a) but a reliance on passive diffusion often results in low recovery (Linhares and Kissinger, 1993b) and in any case sensitive analytical techniques and complex calculations are required to establish drug concentrations (Nourian et al., 2010a). Ultrafiltration also uses small implantable probes and directly samples the interstitial fluid (IF), facilitating direct analysis of drug concentrations without need for complicated homogenisation and extraction processes (Linhares and Kissinger, 1992; Bidgood and Papich, 2002).

Ultrafiltration probes consist of loops of semi-permeable membrane tubing connected to a single collection tube. Once placed in the tissue, a vacuum draws IF across the probe membrane. The

* Corresponding author. Tel.: +61 7 5460 1799.
E-mail address:
c.underwood1@uq.edu.au (C. Underwood).

http://dx.doi.org/10.1016/j.tvjl.2014.05.007
1090-0233/

recovery of small molecules is typically >95% (Linhares and Kissinger, 1992). Following implantation, ultrafiltration probes can be used in unrestrained animals over extended time periods (Linhares and Kissinger, 1992). Pharmacokinetic analyses have been conducted using ultrafiltration probes in equine bone marrow and subcutaneous tissues (Linhares and Kissinger, 1992, 1993b; Spehar et al., 1998; Davis et al., 2005, 2006; Parra-Sanchez et al., 2006), canine subcutaneous tissues (Bidgood and Papich, 2002, 2003, 2005), rodent subcutaneous tissues (Linhares and Kissinger, 1992, 1993b), porcine intramuscular, subcutaneous and intrapleural tissues (Messenger et al., 2011) and bovine subcutaneous tissues (Davis et al., 2007).

Ultrafiltration probe implantation in equine digital lamellar tissue is challenging and potentially painful. The stratum medium of the hoof wall and adjacent lamellar tissue are essential for weight bearing (Pollitt, 2010) hence excessive removal must be avoided. The rate of fluid collection is proportional to probe membrane length (BASi, 2012). Therefore, the probe must be oriented proximodistally to optimise fluid collection. As most experimental laminitis models develop over a 72 h period (van Eps and Pollitt, 2006; de Laat et al., 2010), it is necessary that the probes function without developing infection or causing pain over that time period.

The primary aim of the present study was to develop a technique for ultrafiltration probe placement in, and collection of IF from, lamellar tissue. The objectives were (1) to establish whether ultrafiltration probes could be placed in a proximodistal direction within the lamellar tissue; (2) to establish whether ultrafiltrate could be collected in sufficient volume for ultra-performance liquid chromatography (UPLC) or high-performance liquid chromatography (HPLC) analysis over a 96 h period; (3) to assess whether probe placement caused discomfort and/or lameness; (4) to assess whether ultrafiltrate constituents varied with time; and (5) to establish whether biochemical analytes in the ultrafiltrate differed from plasma.

Materials and methods

Development of probe placement technique

To establish the maximum probe length that could be accommodated in the lamellar tissue, the proximodistal extent of the lamellar tissue was measured at the dorsal midline on sagittal sections of six Standardbred cadaver limbs. A further 15 cadaver limbs were used to develop the placement technique. The hoof wall was resected to the white line at the dorsal, distal toe. A custom-made introducer containing a chisel tip stylet (Fig. 1) was inserted into the lamellar layer at the centre of the resection. The introducer was advanced proximally through the lamellar tissue for a distance equivalent to the length of the lamellae. The chisel tip stylet was replaced with a curved tip stylet and the introducer was advanced until it exited the skin. The curved tip stylet was removed and 3 mL of coloured polyurethane (Plasticast, Dalchem) were injected through the introducer as it was withdrawn. The feet were sectioned to visualise the polyurethane.

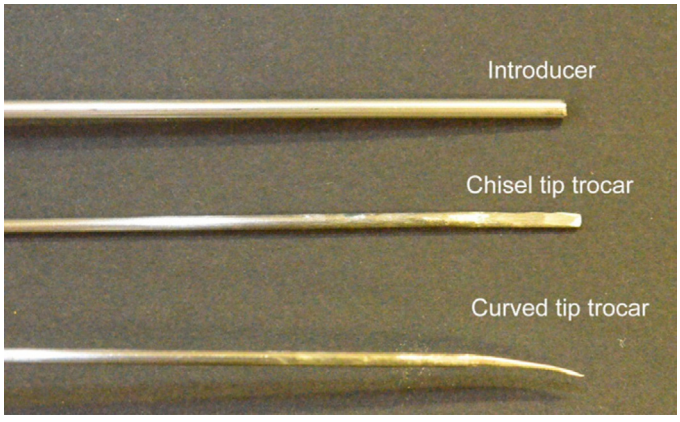

Fig. 1. Photograph showing the custom-made introducer, the chisel tip stylet used when the introducer is passed through the lamellar tissue and the curved tip stylet used to pass the introducer through the skin at the coronary band.

In vivo ultrafiltration probe placement

Six clinically normal Standardbred horses (4–12 year old geldings) with healthy feet and no lameness were used in this part of the study. The animals were housed in stalls, free to move and had ad libitum access to food and water. All procedures were approved by The University of Queensland Animal Ethics Committee (approval number SVS/027/11/TRUST/RIRDC).

Ultrafiltration probes were aseptically soaked in sterile distilled water for 12 h prior to placement, according to the manufacturer's guidelines. The hoof was cleaned and trimmed then soaked for 12 h in a povidone–iodine (PVP-iodine, Vetsense) poultice. The hoof wall was resected (20 × 20 mm) to the white line at the dorsal toe. Perineural analgesia was performed by injecting 3 mL 2% mepivacaine hydrochloride (Mepivacaine Injection, Naturevet) at the distal aspect of the second and fourth metacarpal bones and at the proximal sesamoid bones. The distal limb and hoof were aseptically prepped then wrapped in an antimicrobial surgical incise drape (Ioban, 3M). The limb was extended cranially on a hoof stand. The introducer was inserted as described in cadaver limbs. Once the curved tipped stylet was withdrawn, a custom-made 3–8 ultrafiltration probe (Bioanalytical Systems) was threaded distoproximally through the introducer.

When the probe membrane was positioned at the mid lamellar level the introducer was withdrawn, leaving the probe in the lamellar tissue, with the tubing exiting at the coronary band. The resection site was packed with sterilised dental putty (Coltene Lab Putty, Coltene). The foot was bandaged and placed in a hoof boot (SIMPLE Boot, Cavallo Horse and Rider). The ultrafiltration tubing was connected to a 3 mL glass Vacutainer (Bioanalytical Systems). The Vacutainer holder was taped to the metacarpus and covered with a stable bandage. The Vacutainers were changed every 12 h. The rate of fluid collection was calculated for each 12 h period. Blood was collected 96 h post implantation (end of night 4). Plasma and ultrafiltrate samples were stored at –80 °C prior to analysis. The probes were maintained for 14 days in two animals, and for 4 days in four animals to simulate the maximum length of time required for development of experimental acute laminitis (van Eps and Pollitt, 2006; de Laat et al., 2010).

Pain and lameness evaluation

A complete physical examination including pain evaluation was performed twice daily throughout the experimental period. Lameness was assessed as part of the pain evaluation. Lameness assessment included observation of the horses when unrestrained in the stall and at the walk on a concrete surface. The pain evaluation was based on a previously reported numerical scale of various pain behaviours (van Eps, 2010). Horses were scored from 1 to 4 based on their heart rate, lameness, attitude, appetite, recumbency, and non-specific pain behaviours e.g. teeth grinding. The total score for all pain evaluation parameters in each horse at each observation period was calculated by adding the scores of each parameter (maximum 24, minimum 6).

Fluid analysis

Biochemical analyses were performed on ultrafiltrate collected between 12 and 24 h post implantation (night 1), ultrafiltrate collected between 84 and 96 h post implantation (night 4) and plasma collected at 96 h post implantation (the end of night 4) using a chemistry analyser (Beckman Coulter AU400). The concentrations of total protein (TP), albumin, globulin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), cholesterol, gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), creatine kinase (CK), total bilirubin, carbon dioxide (CO_2), creatinine, glucose, triglycerides, urea, sodium, chloride, potassium, calcium, magnesium and phosphate in plasma were measured in triplicate using the plasma application, with plasma control and calibration samples (Olympus Life and Material Science Europa), as per the laboratory's standard method for analysing equine clinical chemistry samples.

Due to the lack of calibration and control samples in IF, cerebrospinal fluid (CSF) and urine protocols were used for measuring the concentrations of analytes in ultrafiltrate. Ultrafiltrate TP and glucose were measured using the CSF application with CSF calibration and control samples (Olympus Life and Material Science Europa). Ultrafiltrate creatinine, urea, calcium, magnesium, sodium, potassium and chloride were measured using the urine application with urine calibration and control samples (Olympus Life and Material Science Europa). The remaining analytes in ultrafiltrate were analysed using plasma calibration samples. All ultrafiltration samples were measured in triplicate providing sufficient fluid volume was collected.

Tissue collection

At the end of the study each horse was euthanased with pentobarbital sodium (20 mg/kg IV). The forefeet were removed by disarticulation at the pastern. Sagittal sections were cut with a band saw leaving a 1 cm margin either side of the ultrafiltration probe. The dorsal lamellae were dissected from the hoof and distal phalanx. The sagittal sections were cut transversely into six segments, along the length of the