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Comparison of microdialysis sampling perfusion fluid components on the foreign body reaction in rat subcutaneous tissue

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ABSTRACT

Microdialysis sampling is a commonly used technique for collecting solutes from the extracellular space of tissues in laboratory animals and humans. Large molecular weight solutes can be collected using high molecular weight cutoff (MWCO) membranes (100 kDa or greater). High MWCO membranes require addition of high molecular weight dextrans or albumin to the perfusion fluid to prevent fluid loss via ultrafiltration. While these perfusion fluid additives are commonly used during microdialysis sampling, the tissue response to the loss of these compounds across the membrane is poorly understood. Tissue reactions to implanted microdialysis sampling probes containing different microdialysis perfusion fluids were compared over a 7-day time period in rats. The base perfusion fluid was Ringer's solution supplemented with either bovine serum albumin (BSA), rat serum albumin (RSA), Dextran-70, or Dextran-500. A significant inflammatory response to Dextran-70 was observed. No differences in the tissue response between BSA and RSA were observed. Among these agents, the BSA, RSA, and Dextran-500 produced a significantly reduced inflammatory response compared to the Dextran-70. This work demonstrates that use of Dextran-70 in microdialysis sampling perfusion fluids should be eliminated and replaced with Dextran-500 or other alternatives.

1. Introduction

Microdialysis sampling is a widely used *in vivo* collection technique that has been used for more than 35 years for numerous life science applications (Muller, 2013; Robinson and Justice, 1991; Westerink and Cremers, 2007). This diffusion-based separation method uses an isotonic perfusion fluid that flows through inlet tubing into an inner cannula, the inner fiber lumen of a semi-permeable membrane, an outer cannula, and exits via an outlet tube where the dialysate is collected (Fig. 1). These devices are then implanted into tissue allowing collection of solutes from the extracellular fluid (ECF). The solute concentration gradient that exists between the perfusion fluid inside the probe and the surrounding ECF allows analytes smaller than the membrane molecular weight cutoff (MWCO) to diffuse into the membrane lumen to be collected and then quantified (Nandi and Lunte, 2009). The primary reasons for the success and variety of biomedical applications of microdialysis sampling include (1) it is minimally invasive allowing collections to be performed from targeted tissue sites in awake and freely-moving animals as well as in human subjects; (2) it provides analytically-clean samples that require either no or minimal sample preparation allowing a wide variety of chemical analysis schemes to be applied (Davies et al., 2000); (3) it reduces animal numbers since the animal in which the probe is implanted serves as its own control.

Microdialysis sampling was originally developed to collect small hydrophilic molecules such as the catecholamine and amino acid neurotransmitters. With the advent of commercially-available high MWCO membranes incorporated into microdialysis probes, it is now possible to collect peptides and proteins of biological significance including cytokines (Ao and Stenken, 2006; Clough, 2005; Nakamura et al., 1990). This has opened a wide range of possibilities for researchers investigating multiple disease states in different tissues that are believed to incur dysregulated cytokine function (Angst et al., 2008; Ao and Stenken, 2006; Clough et al., 2007; Garvin and Dabrosin, 2003; Helmy et al., 2011; Mellergard et al., 2008; Nielsen et al., 2009; Sjögren et al., 2012).

Prior to the use of high MWCO membranes during microdialysis sampling, it was common practice to use a saline solution, such as Ringer's or Ringer's-Krebs, as a perfusion fluid since these solutions contain a balance of different ions (Na^+, K^+, Ca^{2+} and Cl^-) similar to concentrations existing in the ECF (Benveniste and Huttemeier, 1990). When Ringer's solution is the perfusion fluid through a high

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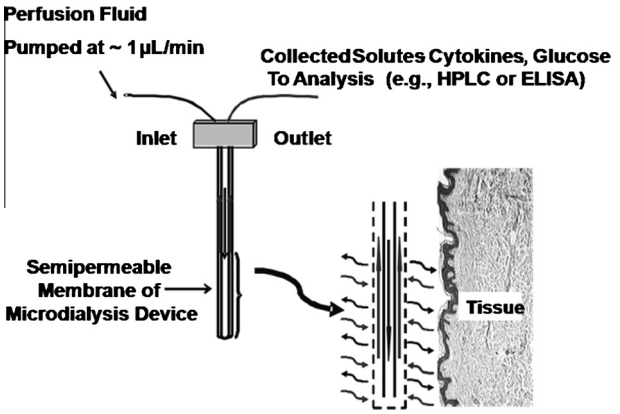

Fig. 1. Microdialysis probe schematic.

MWCO membrane, a significant reduction in expected fluid volumes can be observed due to a difference in hydrostatic pressure causing the perfusion fluid to leak through the membrane pores. This phenomenon is called ultrafiltration and would be expected for high MWCO ultrafiltration membranes, which is defined as any membrane with a MWCO of greater than 50 kDa.

Microdialysis sampling of large bioactive proteins including cytokines can be fraught with two major difficulties – ultrafiltration and non-specific adsorption to the device materials. Ultrafiltration is problematic because fluid is lost across the membrane into the tissue resulting in lower than expected sample volumes. This causes difficulties with chemical analysis techniques such as ELISA that have defined volume specifications. Additionally, the loss of fluid into the tissue space and its effect on tissue physiology is poorly understood. Non-specific adsorption is problematic especially with bioactive proteins since their concentrations are often in the pg/mL range. If non-specific adsorption is not reduced by inclusion of albumin as a blocking protein, proteins in such low concentrations may adsorb to the probe materials precluding their quantitation in dialysates. When using microdialysis membranes with 100 kDa or greater MWCO, colloids (high molecular weight dextrans, albumin, or a combination of the two) are added to the perfusion fluid to reduce ultrafiltration via an increased solution osmotic pressure within the membrane lumen (Hillman et al., 2005a; Rosdahl et al., 1997). Bovine serum albumin (BSA) and human serum albumin have also been used to reduce ultrafiltration and non-specific adsorption (Helmy et al., 2009; Trickler and Miller, 2003).

An unexplored area of research is whether these added colloids to the microdialysis perfusion fluid affect the surrounding tissue in deleterious ways. Fluid loss across the membrane may cause edema or other tissue damage. Albumin (~66 kDa), dextran-60, and dextran-70 can theoretically diffuse through the 100 kDa MWCO into the tissue. Bovine serum albumin is commonly used in rat studies since it can be procured at a significantly lower cost than rat serum albumin. However, there are considerable homology differences between these two albumins that could lead to the potential for immune response.

To date no studies have been performed to determine the effects of commonly used perfusion fluid reagents, i.e. Ringer's solution, Dextran-70, or BSA, on tissue surrounding implanted microdialysis probes. Likewise, it has not been determined if there are differences in the tissue reactions to perfused microdialysis probes vs. non-perfused (simply implanted) probes. These issues related to microdialysis sampling are critical to elucidate since microdialysis sampling is being widely used to collect cytokines and other bioactive proteins involved with inflammatory disease states in animals and human subjects. In this work, we demonstrate that a significant portion of the trauma caused at the site of a microdialysis probe appears to be due to the perfusion fluid agents rather than the implantation process.

2. Materials and methods

2.1. Chemicals

The following chemicals were used in this study: bovine serum albumin (BSA) (Rockland Immunochemicals, Gilbertsville, PA); Dextran-70 and Dextran-500 (Sigma Aldrich, St Louis, MO); Ethylene oxide (Anderson Sterilizers, Inc, Haw River, NC); formalin, 10% and neutral buffered (BDH, VWR, West Chester, PA); HPLC grade water (Fisher Scientific, Waltham, MA); isoflurane (Abbott Laboratories, North Chicago, IL); povidone-iodine (Professional Disposables International Inc, Orangeburge, NY); and Rat Serum Albumin (RSA) (Sigma Aldrich, St Louis, MO). Ringer's solution contained 150 mM NaCl, 5.4 mM KCl, 2.3 mM $CaCl_2$, pH 7.4 and was prepared in HPLC-grade water. All other chemicals were reagent-grade or higher.

2.2. Microdialysis sampling

All microdialysis sampling procedures were performed using CMA 20 microdialysis probes with polyethersulfone (PES) membranes, 100 kDa MWCO and 10 mm length (Harvard Apparatus, Holliston, MA). Prior to implantation, microdialysis probes were sterilized using ethylene oxide (Anderson Sterilizers, Inc, Haw River, NC). The probes are perfused using a BAS Bee pump with appropriate syringes (Bioanalytical Systems Inc., West Lafayette, IN). After completion of the surgical procedures to implant the microdialysis probe, the animal is placed into a CMA 120 freely moving animal collection system (CMA Microdialysis, Solna, Sweden[1]).

2.3. Surgical procedures

Male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN) in the weight range of 250–400 g were used and housed in a climate controlled room at 72 °F. Prior to surgical procedures, rats were allowed access to both food and water *ad libitum*. All animal experiments were approved by the University of Arkansas Institutional Animal Care and Usage Committee (IACUC) and were in compliance with the NIH standards for the ethical treatment of animals.

Rats were anesthetized in an induction chamber with 5% isoflurane in 0.8 L/min oxygen. The rat was then maintained on a nose cone via 2.5% isoflurane in 0.4 L/min oxygen during probe implantation. During the surgical procedure the body temperature was maintained using a CMA 150 temperature controller (CMA Microdialysis, Solna, Sweden). Surgical procedures were performed using aseptic technique. All surgical tools were autoclaved prior to use. The surgical site was shaved and then swabbed with povidone-iodine prior to any incisions.

To implant the microdialysis probe, a '⊥' shaped incision was made into the posterior dorsal subcutaneous tissue followed by a '-' shaped incision made near the base of the neck. Both incisions were about 0.5 cm in length. An autoclaved straw was then passed through the subcutaneous tissue from the posterior to anterior incisions. The tubing lines of the microdialysis probe were then run from the posterior to anterior end of the straw such that outlet lines were located on the anterior side of the rat. The straw was then removed from the animal. A plastic introducer that has split tubing (Harvard Apparatus, Holliston, MA) was then placed subcutaneously at the posterior incision site. The microdialysis probe was then placed in the introducer and the introducer was removed

[1] CMA Microdialysis is now owned by Harvard Apparatus, Holliston, MA, USA.