Subcutaneous microdialysis in the awake rat

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Abstract

Background. Microdialysis is a valued method to study the pharmacokinetics of drug distribution from an injection site or to a tumor site. The objective of this study was to assess the feasibility of prolonged use of subcutaneously implanted microdialysis catheters to assess local retention and systemic uptake of carboplatin in rats post anesthetic recovery.

Methods. Twelve rats were used: one control rat and 11 treatment rats. The control rat was used to outline the surgical implantation technique of the microdialysis catheter. Treatment rats were administered 5mg carboplatin in poloxamer 407 and divided in two treatment cohorts. Microdialysis catheters were used in both cohorts to collect extracellular fluid. Placement technique was modified between the two cohorts. Treatment cohort 1 (n=6) had the microdialysis catheter implanted in the wound bed, sutured in place and tunneled subcutaneously along the dorsum to exit dorsally in the cervical area. The ends were secured to a Velcro strip sutured to the dorsal cervical skin to avoid trauma by the rat. Treatment cohort 2 (n=5) had the catheter looped around the right hind limb, with the functional part sutured in place in the wound bed, and tunneled through a subcutaneously implanted sterile intravenous line exiting dorsally in the cervical area. The ends were maintained within the IV line and held in place with wound clips.

Results. All 11 microdialysis catheters in the treatment cohorts were functional immediately after implantation, but only one out of 11 microdialysis catheters in the treatment cohorts was functional at 24hrs, and none at 48hrs.

Discussion. The results of this study suggest that microdialysis catheters cannot be maintained in unanesthetized rats without further modification of the implantation protocol.
Introduction

Microdialysis (µD) catheters can be a useful tool to collect extracellular fluid (ECF) at the drug delivery sites, allowing an accurate assessment of the rate of local delivery of the drug. Micodialysis has been used for short-term collection of tissue fluids (Johansen, 2002; Zamboni, 2002) but to the author’s knowledge, this has not been used to assess sustained delivery of subcutaneously delivered carboplatin in an awake rodent model.

Carboplatin is a platinum chemotherapeutic agent that is used to systemically treat a variety of tumors in companion animals, such as osteosarcoma and apocrine gland adenocarcinoma of the anal sac (AGASACA) (Ross, 1991; Bennett, 2002; Emms, 2005; Saam, 2011; Simcock, 2012; Selmic, 2014; Kozicki, 2015; Skorupski, 2016; Wouda, 2016) and has been shown to be stable in poloxamer carriers (Exner 2005; Hsu, 2014) and fully release carboplatin (Risselada, 2016) however information is not available about the stability of the combination of carboplatin and poloxamer gel in vivo and its release profile.

The aim of this study was to assess the feasibility of maintaining µD catheters in the awake rat for prolonged collection of ECF.

Materials & Methods

The study was designed using a subcutaneous perineal implantation site, similar to the location of AGASACAs.

Recovery of carboplatin from poloxamer gel (25% poloxamer 407, Professional Compounding Centers of America, Houston, TX) using µD catheters was first assessed in vitro. Three ml of a 5mg/ml carbo-poloxamer compound was maintained in a 37.5°C (rat body temperature) waterbath. A 30,000 MWCO catheter (LM-5 Linear microdialysis catheter, BASi
Inc., West Lafayette, IN) with a 320µm OD was placed in the carboplatin- poloxamer compound. Perfusion was performed daily for 7 days according to standard protocols (Chaurasia, 1999; Konings, 2009) using Lactated Ringers Solution (LRS, Hospira Inc, Lake Forrest, IL).

Perfusate (LRS) was collected over a 45 min period, after a 30 min equilibration period which also allowed the removal of residual fluid. The flow rate was 1.5 µL/min, and the samples were immediately stored at -80°C for batch analysis at a later date. Carboplatin in the samples of the in vitro recovery was measured using high pressure liquid chromatography (HPLC) and a method developed in our laboratory. The carboplatin official analytical reference standard (100 mg) was obtained from US Pharmacopeia (Pharmacopeia (Rockville, MD, USA). The potency of the reference standard was 0.999 mg per mg of material. The carboplatin standard was weighed and dissolved in 100% HPLC-grade distilled water to a concentration of 1 mg/mL.

In all rats, a right perineal subcutaneous incision was made under general anesthesia (Isoflurane (Piranal Healthcare, Piranal Enterprises Ltd, Andhra Pradesh, India) in 100% O2 for box induction and mask maintenance), with a large enough subcutaneous pocket to hold 1ml of a carboplatin in poloxamer 407 compound after which the incision was sutured routinely. The chemotherapeutic agent and the location of the implantation site were chosen as part of a different study investigating the use of local treatment for residual disease in anal sac apocrine gland adenocarcinomas. Analgesia was provided by administration of meloxicam Q 24hrs (1mg/kg PO or SC)(Metacam, Boehringer Ingelheim, St Joseph, MO) the day prior, the day of and the day following surgery, and an additional injection of buprenorphine (0.03mg/kg SC) (Endo Pharmaceuticals, Spring Valley, NY) was provided prior to recovery.

Two implantation techniques were used: in the first group (n=6), the rats (Figure 1) received a microdialysis (µD) catheter (LM-5 Linear microdialysis catheter, BASi Inc., West
Lafayette, IN) was surgically implanted in the right perineal subcutaneous tissue, secured with an encircling suture in the wound (4-0 poliglecaprone 25 (Monocryl, Ethicon Inc, Somerville, NJ) and tunneled subcutaneously to exit dorsally in the cervical area (Figure 1).

Figure 1: Control group. Instrumentation of a rat from the control group is shown, with the micro dialysis catheter (µD) exiting the cervical incision. ‘a’ indicates the end where the dialysate is infused, and b’ the end where the dialysate is collected.

The external portion of the catheter was looped through a perforated Velcro strip. The Velcro strip was secured in place by suturing it to the adjacent skin. The terminal parts of the catheter were each individually tunneled through one of two approximately 3cm long 6F feeding tubes (Argyle™ Feeding Tube, Covidien llc, Mansfield, MA) that were attached to the skin with clips. Next the catheter ends were then looped around in an 180degree turn and each terminal portion fed back into the other feeding tube, secured in place and protected with water proof tape (µD group) (Figure 2).
Figure 2: Microdialysis group. A rat from the µD treatment cohort is shown, fully instrumented with the microdialysis (µD) catheter (a) in place and the right sided perineal wound sutured. Dialysate is recovered in a vial (b). The vascular access port is visible (c). Both ends of the µD catheter were held in place in a 5F catheter attached with wound clips (d) and were taped together during recovery of the rat to prevent traumatic or accidental dislodgement.

In the second group (n=5), the µD catheter was gently looped subcutaneously around the right hind leg to avoid inadvertent damage from very focal traction of the catheter on the anchoring suture. The functional portion of the µD catheter was sutured into the wound bed. A 7 cm long, sterile intravenous (IV) fluid line (Baxter, Deerfield, IL) was placed by bluntly tunneling through the subcutaneous tissues from the wound bed to exit dorsally cranial to the scapulae. The IV line was placed in such a way that the distal end just reached the dorsal edge of the proposed wound bed. The IV line was anchored to the skin with a perforating suture in 4/0 SS (Ethicon Inc, Somerville, NJ). The free ends of the µD catheter were routed through this IV line to exit dorsally in the cervical area (Figure 3) (µD-IV group). The ends were pushed back into the exiting portion of the IV line. The IV line was then externally compressed using sterile skin clips to keep the µD catheter in place, while the IV line itself was closed with waterproof tape. Microdialysis was performed in all rats at time of µD catheter placement and after recovery using LRS at a rate of 1.5 µl/min for a 30 minute period.
Figure 3: Microdialysis group with IV modification. A rat from the µD-IV treatment cohort is shown. The subcutaneously tunneled IV tube (a) is shown with the two ends of the µD catheter (b) exiting the cranial most extent. The IV tube was sutured in place using 4/0 SS suture with a bite through the tubing (using a hypodermic needle). The ends of the µD catheter were looped back into the IV tube and taped in place using waterproof tape.

The study was approved by the Institutional Animal Care and Use Committee at North Carolina State University (#14-080-B). Twelve purpose bred Sprague Dawley rats (Charles River, Wilmington, MA, US) were used in the study (Charles River, Wilmington, MA).

**Results**

In vitro recovery using the µD catheter over 7 days ranged between 166.25 and 201.72 µg/ml, with a relative recovery of the original compound carboplatin concentration ranging between 3.33 and 4.03% (Table 1).

Microdialysis was successfully performed in all rats at time of µD catheter placement using LRS at a rate of 1.5 µl/min for a 30 minute period and a dialysate sample was obtained in all 11 rats. In three rats the ends of the µD catheters were exposed and in 8 rats, the µD catheters were in place and macroscopically intact at the first postoperative microdialysis time point (24hrs). A full dialysate sample could only be recovered in one rat and a couple of drops
insufficient for analysis was obtained in a second rat. The remainder of the µD catheters were nonfunctional (n=9). No dialysate could be obtained for any of the µD catheters at 48 hrs. The ends were trimmed short in the nonfunctional catheters at 24 or 48hrs but allowed to remain in place for 7 days. The intended dialysis area of the µD catheter did not migrate away from the wound bed (Figure 4).

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<th>Relative recovery (%)</th>
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<td>Day 7</td>
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Table 1. In vitro carboplatin recovery data are shown. The starting concentration was 5 mg/ml (5,000 µg/ml). The calculated recovery shows the absolute amount of carboplatin captured in the dialysate, while the relative recovery shows the amount expressed as a percentage of the starting concentration. Breakdown over time shows that there was very little degradation of carboplatin over a 7 day period when kept at body temperature (37.5° C).

Figure 4: Woundbed at necropsy. A. A rat from the µD cohort is shown during necropsy. The µD catheter (a) can be seen in the subcutaneous tissue. B. A rat from the µD-IV cohort is shown during necropsy. The µD catheter can be seen exiting the IV tubing and continuing in a gentle loop in the subcutaneous tissues around the right hind limb.
Discussion

The *in vitro* relative recovery of carboplatin from 37°C poloxamer copolymer gel through a µD catheter remained constant over a 7 day period, indicating that there would be minimal degradation of carboplatin over time at body temperature, and carboplatin was quantifiable when collected via a µD catheter.

Initial recovery of LRS through the µD catheter was at a constant flow, similar to *in vitro* recovery, ensuring proper placement of the µD catheter without obstruction of flow by the perineal placement and anchoring.

The IV line modification was created between cohorts 1 and 2 due to µD catheter malfunction, presumably due to trauma and traction, and was an attempt to protect the µD catheter itself from traction. The anchoring point in the initial cohort was one suture, whereas the catheter was held in the perineal site by being looped around the left hind leg, which would be a less acute angle and with the traction pressure spread out, rather than being focused at the suture anchor.

The rats with the implanted IV line had a subjective increase in tissue inflammation at necropsy (day 7) than the rats with only a µD catheter. Fibrinous tissue could be seen at the termination point of the IV line during necropsy, but this did not compress the catheter. The wound bed more distally appeared less inflamed, leading to the conclusion that the reaction was due to the IV line, rather than the µD catheter.

Conclusion

The authors conclude that subcutaneously implanted µD catheters cannot be maintained in the awake rat without further modification of the techniques used in this study.
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References:


