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The development of multiple probe microdialysis sampling in the stomach

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ABSTRACT

A multiple probe approach of implanting microdialysis probes into each separate tissue layer would better represent sampling from the stomach. Presently, microdialysis sampling experiments are performed with only a single probe in the submucosa to represent sampling from the stomach tissue. The focus of this research was to develop a four-probe microdialysis sampling design to simultaneously monitor the stomach lumen, mucosa, submucosa and in the blood of the rat. Due to the small outer diameter of the microdialysis probe (350 μm), implantation into each separate layer was achieved with confirmation of probe location from histological examination. To assess the significance of sampling by this approach, multiple probe microdialysis sampling was used to monitor drug absorption in the stomach. Salicylic acid, caffeine and metoprolol were individually dosed to the ligated stomach. Analysis of the dialysate samples was performed by HPLC–UV and concentration–time curves and pharmacokinetics analysis were used to determine differences between the different probe locations.

1. Introduction

Microdialysis sampling has proven to be a successful technique in numerous pharmacokinetics studies to site-specifically monitor drug concentrations in several tissues. In most of these studies, sampling is done by a single or dual probe approach where a probe is implanted in the target tissue and, in some cases, also in the blood for comparison [1–4]. Microdialysis sampling from a single probe in a homogeneous tissue is generally regarded as a good representation of concentrations from the whole tissue. However, a study of implanting multiple probes in the median lobe of the liver suggested small regional differences observed between implanted probes [5]. Therefore, even more so in tissues that consist of different layers (i.e. heterogeneous tissues), microdialysis sampling by a multiple probe approach in each tissue layer is expected to be a more accurate approach to monitoring tissue concentrations.

The stomach is a heterogeneous tissue where a multiple probe approach can be used to monitor the different layers, in particular in the stomach lumen, mucosa and submucosa. Microdialysis sampling in the stomach is presently performed with a probe implanted only into the submucosa. Most recently, stomach submucosal microdialysis sampling was used to study histamine release from enterochromaffin-like (ECL) cells [6–9]. ECL cells are; however, located in the mucosa layer while the microdialysis probes were implanted in the submucosa layer. Kitano et al. discussed that sampling in the submucosa may not represent the exact amount of histamine present since degradation can occur as histamine diffuses from the mucosa to the submucosa and also to the probe [10]. Therefore, the ability to monitor in both the mucosa and submucosa would enhance the aforementioned studies and also further expand *in vivo* sampling of the stomach.

The purpose of this research was to extend the use of microdialysis sampling in the stomach by developing a four-probe design of simultaneously implanting microdialysis probes in the stomach lumen, mucosa, submucosa and in blood of the rat. To our knowledge, this is the first presentation of a multi-probe approach of microdialysis sampling in both the stomach mucosa and submucosa. To determine the significance of sampling from the different locations in the stomach, this four-probe design was used to monitor drug absorption through the ligated rat stomach. After probe implantation methods were developed, test compounds with reported differing degrees of absorption through the stomach were dosed by oral gavage. Salicylic acid (SA), caffeine and metoprolol were chosen as test compounds for this study based on the reported high, moderate and low absorption of these compounds through the rat stomach, respectively [11,12]. Differences in the observed concentrations in each studied region and the extent of drug absorption of the test compounds were used to evaluate the efficiency of this multiple probe approach.

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2. Experimental

2.1. Chemicals and reagents

Caffeine, metoprolol tartrate, salicylic acid (sodium salt) and chemicals for Ringer's and artificial gastric solution were purchased from Sigma (St. Louis, MO). Ringer's solution consisted of 145 mM NaCl, 2.8 mM KCl, 1.2 mM $CaCl_2$ and 1.2 mM $MgCl_2$. Artificial gastric solution (pH 2.5–3.0) consisted of 87.4 mM NaCl, 4.0 mM KCl, 0.8 mM $MgSO_4$, 2.1 mM $NaSO_4$ and 19.3 mM mannitol. Chemicals for the HPLC–UV system were purchased from Fisher Scientific (Fair Lawn, NJ). Water for buffer, Ringer's solution and artificial gastric solution preparation was processed through a Labconco WaterPro Plus water purification system (18 MΩ/cm) (Kansas City, MO) and solutions were filtered through a 47 mm, 0.22 μm nylon filter prior to use.

2.2. Linear probe construction

Linear microdialysis probes were constructed in-house based on previously described techniques where the probe inlet and outlet are connected to the membrane in a successive fashion [13]. The linear probes were used for sampling in the stomach lumen, mucosa and submucosa. The probe membrane was a 5 mm (effective length) polyacrylonitrile (PAN) dialysis membrane (MWCO 40 kDa; 350 μm o.d.; 250 μm i.d.) (Hospal Industrie, Meyzleu, France). Polyimide tubing (223 μm o.d.; 175 μm i.d.) (Microlumen, Inc., Tampa, FL) was used for the probe inlet and outlet. A short piece of tygon microbore tubing (1520 μm o.d.; 508 μm i.d.) (Norton Performance Plastics, Akron, OH) was used as an adaptor to connect the inlet of the probe to a syringe containing perfusate. All probe pieces were connected by UV glue (Ultraviolet Exposure Systems, Sunnyvale, CA) by curing with an ELC-450 UV source (Electrolite Corporation, Bethel, CT). Probes were sealed in a plastic bag and used within 1 week of construction.

2.3. Vascular probe construction

Vascular microdialysis probes were fabricated in-house for implantation in the jugular vein. The vascular probe was constructed in a cannula-style geometry based from a previously described design [14]. The probe consisted of 10 mm (effective length) PAN membrane (MWCO 40 kDa; 350 μm o.d.; 250 μm i.d.) (Hospal Industrie). The membrane was slid over the polyimide tube probe inlet (163 μm o.d.; 122 μm i.d.) (Microlumen, Inc.). A 10 mm piece of MRE-033 tubing (Braintree Scientific, Braintree, MA) was connected to the membrane piece. The polyimide outlet was inserted into the MRE-033 and UV glue was used to close the MRE-033 opening. A 2 cm piece of PE-50 was connected to the MRE-033 tubing to add extra support. A short tygon microbore tubing piece was used as an adaptor to connect a syringe of perfusate to the inlet of the probe. Probes were stored in a sealed plastic bag and used within 1 week of construction.

2.4. Animals and surgical preparation

Female Sprague–Dawley rats (225–300 g) (Charles River Laboratories, Inc., Wilmington, MA) were initially housed with free food and water access on 12-h light/dark cycles in temperature and humidity controlled rooms. The University of Kansas IACUC committee approved all surgical procedures.

In order to clear the stomach of food contents, the rats were fasted prior to experimentation. Rats were placed in a metabolism cage with a rodent Elizabethan collar (Braintree Scientific) affixed around the neck for 15–20 h prior to the experiment. During the fasting procedure, the rat had free access to water *ad libitum*.

After fasting, the rats were pre-anesthetized by isoflurane inhalation. The rats were then given full anesthesia by a 67.5 mg/kg ketamine, 3.5 mg/kg xylazine and 0.66 mg/kg acepromazine cocktail given subcutaneously. A subcutaneous injection of 2 mL of 2.5% dextrose in lactated Ringer's was given as a means of fluid for the rat while under anesthesia. The hair on the abdomen and neck was shaved and the area was scrubbed with 70% isopropyl alcohol. The rat's body temperature was maintained at 37 °C during surgery and throughout sampling by a CMA 150 temperature controlling system (North Chelmsford, MA). Anesthesia was maintained by intramuscular booster injections of ketamine (17 mg/kg).

2.5. Probe implantation in the stomach

The stomach was exposed by a midline incision across the abdomen. The pyloric sphincter was ligated off with 3–0 suture. A gavage tube (MRE-080) (Braintree Scientific) was inserted through the mouth, down the esophagus, and into the stomach. The gavage tube was kept in place by ligation with 3–0 suture near the cardiac sphincter. The end of the gavage tube was connected to an 18-gauge needle that was connected to a 5 mL syringe. The stomach was flushed several times with water until the solution was clear. The stomach was then flushed once with artificial gastric solution. Fresh artificial gastric solution (3 mL) was injected into the ligated stomach. Linear probes were implanted into the lumen, mucosa, and submucosa with the use of a 2-in. 25-gauge needle that served as an introducer. The needle punctured the stomach tissue and was tunneled parallel within the appropriate layer. The probe was inserted into the inside of the needle and only the needle was removed, leaving the probe in place in the tissue. Tissue glue (3M, St. Paul, MN) was used to close the probe entrance and exit sites and to hold the probe in place.

2.6. Probe implantation in the jugular vein

An incision in the neck was made to expose the right jugular vein. Extra tissue was cleaned from the vein until a section of the vein was isolated onto a metal spatula. A small cut was made on the vein with spring scissors (Fine Science Tools, Foster City, CA). The vascular probe was inserted into the jugular vein with the probe membrane directed towards the heart. The jugular vein was ligated in place with 3–0 suture. The probe inlet and outlet were externalized through the incision and the incision was carefully closed with wound clips around the probe inlet and outlet.

2.7. Microdialysis experiment

Four 1 mL Hamilton gastight syringes (Reno, NV) were placed in a CMA model 400 syringe pump. The inlets of the microdialysis probes were connected to the syringes and perfused at 1 μL/min. The outlets of the microdialysis probes were placed into BASi Honey Comb refrigerated fraction collectors (West Lafayette, IN) set to collect samples every 15 min. The microdialysis samples were collected into 250 μL polyethylene microcentrifuge tubes (Fisher Scientific). To initially flush the microdialysis probes, Ringer's solution was perfused through the mucosa, submucosa and vascular probes and artificial gastric solution was perfused through the lumen probe for 1–2 h after implantation.

Calibration of the microdialysis probes was performed *in vivo* by delivery of the analyte. Probe extraction efficiency determination by this method has been previously described in detail [15]. The probes were perfused with 10 μM of the analyte until a steady-state dialysate concentration was achieved (~45 min) then five samples