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The direct comparison of health and ulcerated stomach tissue: A multiple probe microdialysis sampling approach

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ABSTRACT

The ability to directly compare gastric ulcerated and healthy tissue would aid in the understanding of the physiological differences between these tissue types. Presently, these comparisons can only be drawn by the use of separate animal groups, which results in increased study variability. The focus of this research was to develop a four-probe microdialysis sampling approach to directly compare ulcerated and healthy tissue in the same animal. After controlled chemical ulcer induction, probes were implanted in the ulcerated and healthy stomach submucosa, stomach lumen and in the blood. To assess the significance of this multiple probe approach, drug concentrations in each probe location were monitored after selected compounds were dosed to the ligated stomach by oral gavage. Analysis of the dialysate samples was performed by HPLC-UV and concentration-time curves and pharmacokinetics analyses were used to determine differences between these tissue types.

1. Introduction

Diseases of the gastrointestinal (GI) tract can alter the anatomy of the GI tissue resulting in physiological changes in comparison to normal, healthy states. An example of alterations that occurs is that of absorption across the GI tissue. Disease causes variability in absorption where some drugs will exhibit increased absorption while others decrease. This variability is a result of the effects disease has on the surface area and pH levels in the GI tract as well as affects on gastric emptying [1,2]. Peptic ulcer disease is complex in origin in that many physiological and environmental factors contribute to the disease making proper treatment challenging [3]. Ulcers form from the erosion of the mucosa layer, the innermost layer of the gastrointestinal tract. Without this protective barrier, the underlying submucosa is subjected to the harsh environment of the lumen, causing further damage to the tissue [4]. Normally, for drugs to be absorbed from the GI tract, the drug must permeate the mucosa. In the case of ulcers, the mucosa is absent resulting in non-selective and enhanced absorption at the ulcer relative to normal, healthy tissue. Drug absorption studies are usually performed in normal, healthy subjects but in situations such as ulcerations, adjustments to the dose may be needed to compensate for potential increased absorption. Techniques to locally monitor what is happening at the ulcerated tissue relative to healthy tissue could aid in understanding drug bioavailability in relation to disease.

Traditional methods of studying absorption across the stomach include blood sampling, *in situ* closed loop methods and excised tissue studies [5–8]. Blood sampling, although sufficient for measuring overall bioavailability, does not give an assessment of local absorption. *In situ* closed loop methods and excised tissue studies result in local GI measurements, but require an exhaustive number of animals needed for each experiment. The use of these techniques to compare ulcerated and healthy tissue rely on separate groups of animals to draw the necessary comparisons which results in the loss of analysis of subtle differences between these tissue types. Microdialysis sampling is an *in vivo* site-specific technique that has been used in numerous pharmacokinetics studies to monitor drug concentrations in several tissues. Microdialysis sampling is an improvement to the aforementioned techniques since it is site-specific and multiple samples can be taken from the same animal. In addition, due to the small outer diameter of the microdialysis probe, it is possible to implant multiple probes within one tissue. In the stomach, previous studies by the authors demonstrated successful multiple probe microdialysis sampling to simultaneously monitor the different tissue layers.

The goal of this study was to develop a multiple probe microdialysis sampling approach to directly compare ulcerated and healthy stomach tissue in the same animal. After chemical ulcer induction, methods of probe implantation in the ulcerated and healthy submucosa, lumen and the blood of the same rat were developed. To test the significance of this approach, this four-probe design

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was used to monitor absorption across the ulcerated stomach. Test compounds with well characterized absorption through the stomach were dosed by oral gavage and concentrations in each probe location were studied by concentration-time curves and pharmacokinetics analyses. Salicylic acid (SA), caffeine and metoprolol were chosen as test compounds for this study based on their reported high, moderate and low absorption through the rat stomach, respectively [9,10]. To the authors' knowledge, this is the first presentation of microdialysis probe implantation into the gastric ulcer and use of microdialysis sampling to directly compare ulcerated and healthy tissue.

2. Experimental

2.1. Chemicals and reagents

Dose compounds 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 buffers, 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. Microdialysis probe construction

For both the linear and vascular probes, polyacrylonitrile (PAN) dialysis membrane (MWCO 40 kDa; 350 μm o.d.; 250 μm i.d.) (Hospal Industrie, Meyzleu, France) was used for the probe semipermeable membrane and polyimide tubing (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 a connector for the probe inlet and perfusate syringe. Probe pieces were connected by UV glue (Ultraviolet Exposure Systems, Sunnyvale, CA) by curing with an ELC-450 UV source (Electrolite Corporation, Bethel, CT). All probes were sealed in plastic bags and used within one week of construction.

2.2.1. Linear probe

Linear microdialysis probes were constructed in-house based on previously described techniques [11]. The probe membrane was 5 mm (effective length) and connected to the inlet and outlet polyimide tubing (223 μm o.d.; 175 μm i.d.) in a successive fashion.

2.2.2. Vascular probe

Vascular microdialysis probes were constructed in a cannula-style geometry based from a previously described design [12]. The probe consisted of 10 mm (effective length) PAN membrane. This membrane was slid over the polyimide tube probe inlet (163 μm o.d.; 122 μm i.d.). 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.

2.3. Surgical preparation and procedures

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.

2.3.1. Chemical ulcer induction

All tools were autoclaved with a Harvey SterileMax benchtop autoclave. The rats were anesthetized with an isoflurane vaporizer (VetEquip, Inc., Pleasanton, CA) by a 95:5% O_2:CO_2 medical oxygen mix (1–1.5 L/min) and 2–3% isoflurane. The hair on the left side of the rat just below the ribcage was shaved and sterilized with alternating betadine and 70% isopropyl alcohol scrubs.

An incision was made just below the ribcage to expose the stomach. To induce ulceration, 50 μL of 20% acetic acid (v/v) was injected into the stomach submucosa of the ventral side of the antrum [13]. To reduce adhesion of the ulcer with neighboring tissues during the ulcer formation period, a small amount of sterile lubricant (Surgilube, E. Fougera & Co., Melville, NY) was rubbed over the ulcer injection site. After ulcer induction, the muscle and skin were sutured closed and the rat was removed from anesthesia. A subcutaneous injection of 0.1 mg/kg buprenorpherine was given as post-operative care.

2.3.2. Fasting

Two days after ulcer induction, the rats placed in a metabolism cage with a rodent Elizabethan collar (Braintree Scientific) affixed around the neck for 15–20 h prior to the microdialysis experiment. During the fasting procedure, the rat had free access to water.

2.3.3. Microdialysis probe implantation

After fasting, the rats were pre-anesthetized by isoflurane inhalation. The rats were then given full anesthesia by a subcutaneous injection of a 67.5 mg/kg ketamine, 3.5 mg/kg xylazine and 0.66 mg/kg acepromazine mixture. A subcutaneous injection of 2 mL of 2.5% dextrose in lactated Ringer's was given as a means to replenish fluid to 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). Full anesthesia was maintained by intra-muscular booster injections of ketamine (17 mg/kg).

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. Using the gavage tube, the stomach was flushed several times with water until the stomach solution was clear. The stomach was then flushed once with artificial gastric solution followed by a 3 mL injection of artificial gastric solution into the stomach. Linear probes were implanted into the stomach lumen and submucosa of both healthy and ulcerated tissue with the use of a 2-in. 25-gauge needle introducer. The needle punctured the stomach tissue and was tunneled parallel within the tissue. The probe was inserted into the inside of the needle and only the needle was removed, leaving the probe in place. Tissue glue (3M, St. Paul, MN) was used to close the probe entrance and exit sites.

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 probe was ligated in place with 3-0 suture. The probe inlet and outlet were