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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

externalized through the incision and the incision was carefully closed with wound clips around the probe inlet and outlet.

2.4. Microdialysis experiment

The microdialysis probes were perfused at 1 μL/min by a CMA model 400 syringe pump. Dialysate samples were collected every 15 min into 250 μL polyethylene microcentrifuge tubes (Fisher Scientific) in BASi Honey Comb refrigerated fraction collectors (West Lafayette, IN). To initially flush the microdialysis probes (1–2 h), Ringer's solution was perfused through both submucosa probes and the vascular probe and artificial gastric solution was perfused through the lumen probe.

Calibration of the microdialysis probes was performed *in vivo* by delivery of the analyte [14]. The probes were perfused with 10 μM of the analyte until a steady-state dialysate concentration was achieved (~45 min) then five samples were collected for analysis. The calibration solution was flushed from the probes by perfusion with Ringer's or artificial gastric solution for approximately 1.5 h. During this flushing period, samples were collected to check chromatographically for the presence of any remaining analyte in the microdialysate.

When analyte was not detected in the dialysate, the stomach solution was removed by pulling on the syringe connected to the gavage tube. Test compounds (5 mM dissolved in artificial gastric solution) were given by gavage by injection of 3 mL of the dose into the stomach.

2.5. Ulcer index measurement and tissue histology

After completion of the experiment, the rats were euthanized by isoflurane inhalation overdose. The stomach was harvested and cut along the greater curvature. The ulcer index (UI) was measured as the ulcer area (mm^2) from the mucosal side of the stomach. After UI measurement, the stomach was placed in a 10% neutral buffered formalin solution. The sample was taken to the pathology lab at Lawrence Memorial Hospital (Lawrence, KS). At the pathology lab, tissue slices were processed from tissues embedded in paraffin wax. The tissue slices were mounted on microscope slides and stained with hematoxylin and eosin (H and E) dyes. To confirm probe placement in the healthy and ulcerated submucosa, the slides were studied microscopically. The results of the tissue slides were discussed with a pathologist.

2.6. Chromatographic system

All dialysate samples were analyzed by HPLC-UV. The system consisted of a Shimadzu LC-10AD pump, a Shimadzu SPD-10AV UV–vis spectrophotometric detector and a Shimadzu SCL-10Avp system controller. Sample injections were made into a Rheodyne model 7125i injector (underfill of 10 μL into a 25 μL PEEK sample loop). Data was acquired using EZ Start version 7.3 software (Shimadzu).

Separation of SA was achieved on a Phenomenex Gemini RP C_{18} column (150 mm × 2.00 mm, 5 μm particle). The mobile phase consisted of ammonium phosphate (25 mM; pH 2.5)/acetonitrile (75/25, v/v), flow rate = 0.35 mL/min. Detection of SA performed at 300 nm. Separation of caffeine was achieved on a Phenomenex Gemini RP C_{18} column (150 mm × 2.00 mm, 5 μm particle). The mobile phase consisted of sodium acetate (30 mM; pH 4.0)/acetonitrile (90/10, v/v), flow rate = 0.35 mL/min. Detection of caffeine was performed at 280 nm. Separation of metoprolol was achieved on an Agilent Zorbax Bonus RP column (100 mm × 2.1 mm, 3.5 μm particle). The mobile phase consisted of ammonium acetate (25 mM; pH 4.0)/acetonitrile (90/10, v/v), flow rate = 0.30 mL/min. Detection of metoprolol was performed at 275 nm.

Calibration curves were constructed by spiking analyte into Ringer's or artificial gastric solution in the concentration range of 1–200 μM, analyzed in triplicate. Each calibration curve was constructed using Microsoft Excel (Redmond, WA). The method of least squares was applied to determine linearity. The post-dose lumen dialysate samples were diluted 1:5 or 1:10 to maintain the samples in the tested concentration range.

2.7. Data analysis

Concentration-time curves (concentrations corrected for probe extraction efficiency) were generated with OriginLab version 6.0 software (Northampton, MA). Pharmacokinetics parameters were calculated using WinNonlin version 4.1 software (Pharsight, Mountain View, CA). To describe absorption from the lumen and from large ulcer submucosa, a one-compartment, first-order elimination was used (WinNonlin Model 1) [15,16]. Modeling for the healthy and small ulcer submucosa and blood was described by a one compartment, first-order process (WinNonlin Model 3) [16]. The area under the curve (AUC) was determined by the trapezoidal rule. Statistical analysis of the pharmacokinetics parameters was performed with OriginLab software by the one-way ANOVA followed by a Tukey's test to determine differences between the studied sites as well as between SA, caffeine and metoprolol in each site. A level of $p < 0.05$ was considered statistically different.

3. Results

3.1. Analytical method validation

Fig. 1 is an example chromatogram of ulcer submucosa dialysate taken prior to and 15 min after a 5 mM oral dose of SA was given by gavage. The chromatograms exhibit good method selectivity for SA, which was also observed with all analytes and probe locations. Calibration curves were linear over the range of 1–200 μM (goodness of fit of 0.98–1). The intra-assay precision was 97% or greater for all

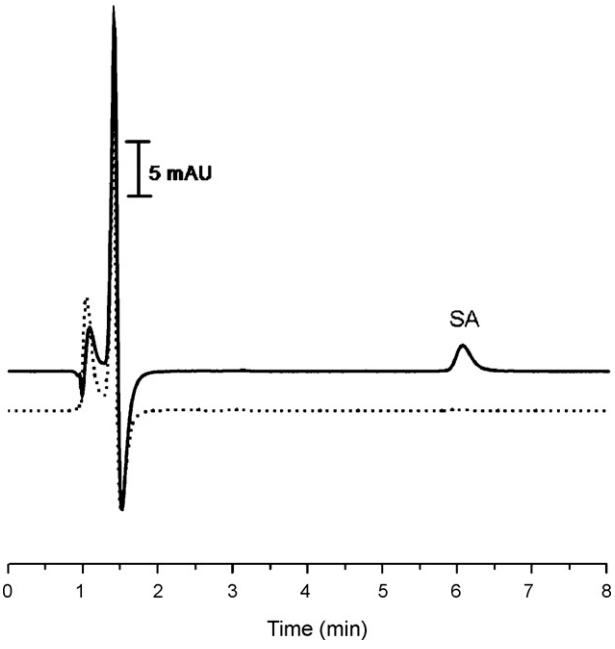

Fig. 1. HPLC-UV chromatograms of ulcerated submucosa dialysate collected prior to dosing (dotted line) and 15 min after a 5 mM SA oral dose (solid line).

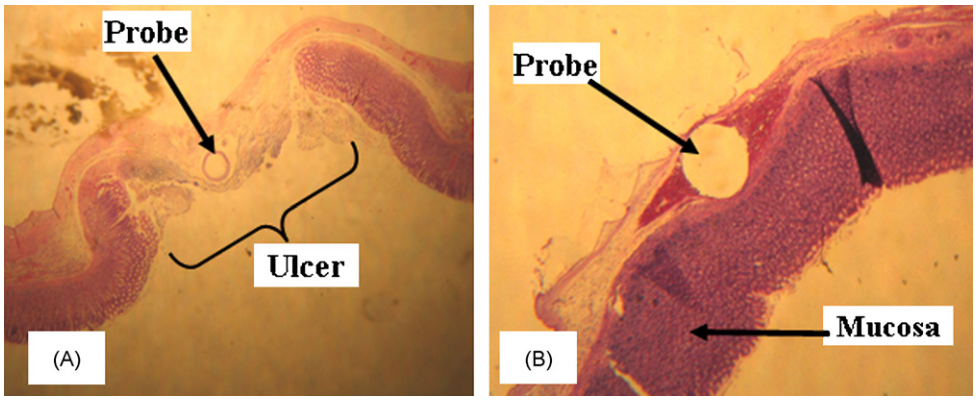

Fig. 2. Stomach tissue histology showing microdialysis probe implantation in (A) the submucosa of an ulcer (20× magnification) and (B) in the healthy submucosa of the same rat (40× magnification).

curves. The limits of detection for SA, caffeine and metoprolol were 200, 100 and 200 nM, respectively.

3.2. Extent of ulceration

After the ulcer formation period (3 days), the ulcer was visible macroscopically from the serosal side of the stomach. The ulcerated tissue appeared white with blood engorgement at the ulcer base. The membrane of the probe was consistently implanted over the visibly white area of the ulcer. Following the method for acetic acid ulcer induction reported by Takagi et al. (without lubricant coating the ulcer), the ulcer would frequently adhere to neighboring liver and adipose tissue. To reduce adhesion, a surgical lubricant was used to coat the ulcer and prevent strong adhesion of the ulcer to neighboring tissues. UI values for ulcers formed with lubricant coated over the induction area were determined to be $17 \pm 4\,mm^2$ ($n = 9$). Ulcers formed without the use of lubricant were $37 \pm 8\,mm^2$ ($n = 9$), twice that of ulcers with lubricant. For these studies, ulcers formed with lubricant were classified as small ulcers in the range of 10–20 mm^2 and ulcers without lubricant were classified as large ulcers in the range of 30–40 mm^2.

3.3. Microdialysis probe location

The stomach tissue histology was processed as described above to verify the location of the probes within both the healthy and ulcerated submucosa. Fig. 2 shows the microdialysis probe implantation sites for both tissue types. Fig. 2A shows a microdialysis probe implanted into the ulcerated submucosa. As illustrated, the mucosa layer is completely eroded and the probe is positioned in the intact submucosa layer. Fig. 2B demonstrates a probe implanted in the healthy submucosa tissue of the same stomach. The underlying mucosa is intact and healthy where the probe was implanted.

Table 1
Extraction efficiency values for probes implanted in the ulcerated rat stomach

	Probe location	Extraction efficiency (%)		
		Salicylic acid	Caffeine	Metoprolol
Large ulcers	Lumen	67.5 ± 4.7	42.0 ± 1.6	37.2 ± 5.9
	Ulcer submucosa	23.9 ± 11.1	31.7 ± 3.4	19.4 ± 2.7
	Healthy submucosa	23.8 ± 3.2	36.7 ± 8.7	20.7 ± 0.45
	Blood	40.9 ± 11.3	43.6 ± 2.6	34.8 ± 4.9
Small ulcers	Lumen	61.3 ± 4.8	39.2 ± 9.2	44.1 ± 15.0
	Ulcer submucosa	23.7 ± 11.6	31.7 ± 3.3	33.3 ± 10.4
	Healthy submucosa	14.2 ± 2.4	33.4 ± 8.1	24.3 ± 5.8
	Blood	40.9 ± 13.2	36.8 ± 12.1	30.1 ± 15.6

Values are average ± standard deviation ($n = 3$ for each value for large ulcers and $n = 4$ for each value for small ulcers).

3.4. Microdialysis probe extraction efficiency

The extraction efficiency values from calibration by delivery in each probe location for each test dose compound are displayed in Table 1 for both small and large ulcers. Even though the environment at the ulcerated tissue is affected by the lack of mucosa to protect it from the lumen, the extraction efficiency values between the healthy and ulcerated submucosa tissue are equivalent. This supports the histological results that the submucosa is still intact in the ulcerated tissue for these studies.

3.5. Direct comparison of healthy and ulcerated tissue

3.5.1. Analysis of concentration-time curves

The results of multiple probe microdialysis sampling in the stomach lumen, submucosa of both healthy and small ulcerated tissue and in the blood after a 5 mM dose of SA, caffeine and metoprolol are shown in Figs. 3–5, respectively. Concentrations of test compounds were detected in all of the studied probe locations by the first 15 min sample when SA or caffeine was dosed. Concentrations were observed in the lumen and

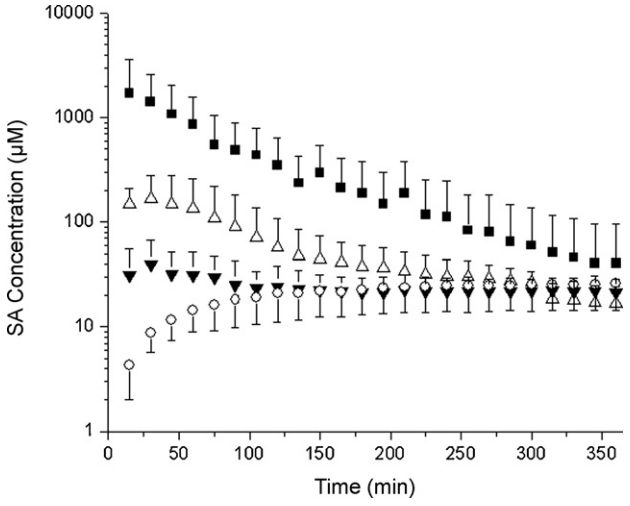

Fig. 3. Plot of the average SA concentration in the lumen (■), submucosa of ulcerated (△) and healthy tissue (▼) and in blood (○) determined through microdialysis sampling as a function of time after a 5 mM oral dose in the ulcerated stomach ($n = 3$).

Table 2
Pharmacokinetics parameters from microdialysis sampling from the ulcerated and healthy stomach submucosa, stomach lumen and the blood in small ulcer studies ($n = 3$)

		k_e (h^{-1})	$t_{1/2}$ (h)	C_{max} (μM)	$AUC_{0-6\,h}$ (μM × h)
Lumen	SA	0.75 ± 0.26	0.64 ± 0.48	2121 ± 1776	2452 ± 1919
	Caffeine	0.32 ± 0.17	2.21 ± 1.55	2968 ± 1189	10909 ± 8908
	Metoprolol	0.021 ± 0.028[a]	213 ± 304	3606 ± 1441	380022 ± 335039
Ulcerated submucosa	SA	0.35 ± 0.23	1.61 ± 0.78	204 ± 132[b]	394 ± 260
	Caffeine	0.34 ± 0.28	11.41 ± 11.62	2000 ± 1689	3822 ± 4239
	Metoprolol	0.034 ± 0.044	16.15 ± 21.70	1774 ± 871	10942 ± 13714
Healthy submucosa	SA	0.030 ± 0.019[c]	14.27 ± 8.51[c,d]	36 ± 14	1067 ± 1388
	Caffeine	0.051 ± 0.082	102 ± 91	44 ± 18[c]	3300 ± 2281
	Metoprolol[e]	0.47	1.93	20	155
Blood	SA	0.0074 ± 0.0093[c]	409 ± 543	22 ± 14	1420 ± 172[a,d]
	Caffeine	0.025 ± 0.021[c]	99 ± 84	45 ± 2[c]	4341 ± 120
	Metoprolol[e]	–	–	–	–

Values are the average ± standard deviation. Statistical analysis by one-way ANOVA followed by a Tukey test. (–) Indicates no modeling done since no concentrations were detected.

[a] Significantly different relative to other drugs ($p < 0.05$).
[b] Significantly different from metoprolol ($p < 0.05$).
[c] Significantly different from the lumen ($p < 0.05$).
[d] Significantly different from the ulcerated submucosa ($p < 0.05$).
[e] No statistical analysis performed.

ulcerated submucosa when metoprolol was dosed. Only one of the rats used for this study had detectable concentrations in the healthy submucosa while the remaining rats exhibited concentrations below detection limits in the healthy submucosa. All rats had no detectable metoprolol in the blood for the metoprolol studies.

Concentrations in the lumen decreased over time when both SA and caffeine were dosed. The decrease was not as rapid when caffeine was dosed indicating less absorption from the stomach when caffeine was dosed relative to SA. Metoprolol concentrations in the lumen remained constant over time, supporting low overall absorption in the stomach. As predicted, concentrations observed in the ulcerated submucosa were consistently greater than observed in the healthy submucosa for all dosed compounds.

3.5.2. Pharmacokinetics parameters analysis

To numerically characterize the plots from Figs. 3–5, pharmacokinetics parameters were generated for each studied probe location as illustrated in Table 2. To describe the overall rate of absorption from the stomach, the lumen parameters were compared for each compound. The rate of elimination (k_e) was in the order SA > caffeine > metoprolol (k_e = 0.75 (SA); 0.32 (caffeine) and 0.021 h^{-1} (metoprolol). The AUC increased with SA the smallest to metoprolol with the highest AUC. Similar C_{max} values in the lumen were observed as expected since the same 5 mM dose was given for each compound. When comparing the ulcerated and healthy submucosa parameters, smaller k_e, $t_{1/2}$, C_{max} and AUC values were determined in the healthy submucosa tissue relative to ulcerated tissue. Parameters observed in the blood were the lowest with dosed SA and caffeine relative to the lumen and submucosa of both healthy and ulcerated tissue. No modeling was performed with metoprolol in the healthy submucosa or the blood since concentrations were below detection limits in the blood and only one rat used in the study had detectable concentrations in the healthy submucosa.

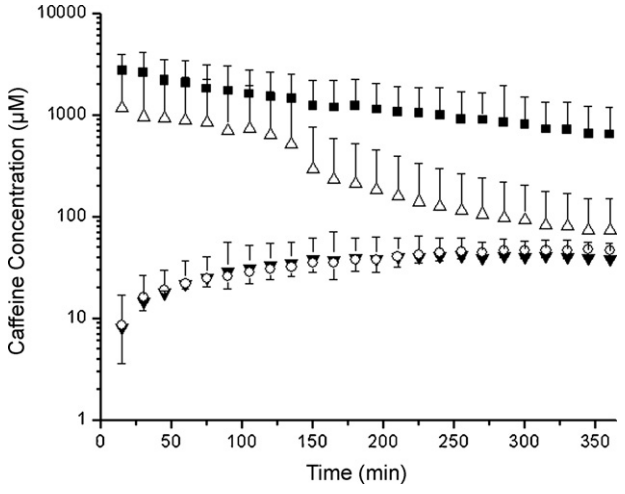

Fig. 4. Plot of the average caffeine concentration in the lumen (■), submucosa of ulcerated (△) and healthy tissue (▼) and in blood (○) determined through microdialysis sampling as a function of time after a 5 mM oral dose in the ulcerated stomach ($n = 3$).

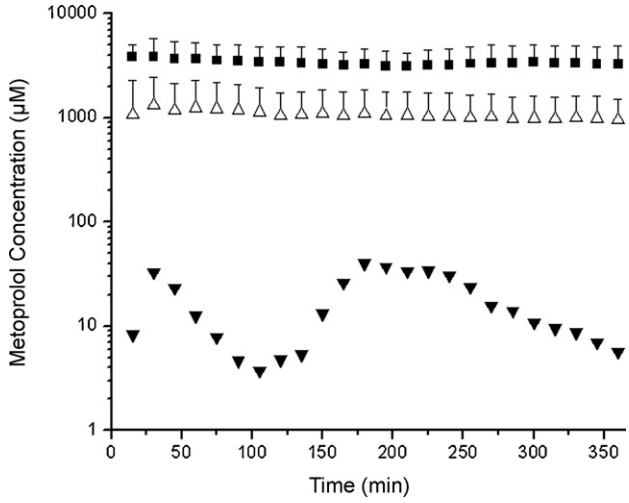

Fig. 5. Plot of the average metoprolol concentration in the lumen (■), submucosa of ulcerated (△) and healthy tissue (▼) determined through microdialysis sampling as a function of time after a 5 mM oral dose in the ulcerated stomach ($n = 3$). Only one of the three rats had detectable drug in the healthy submucosa. All rats had no detectable drug in the blood.

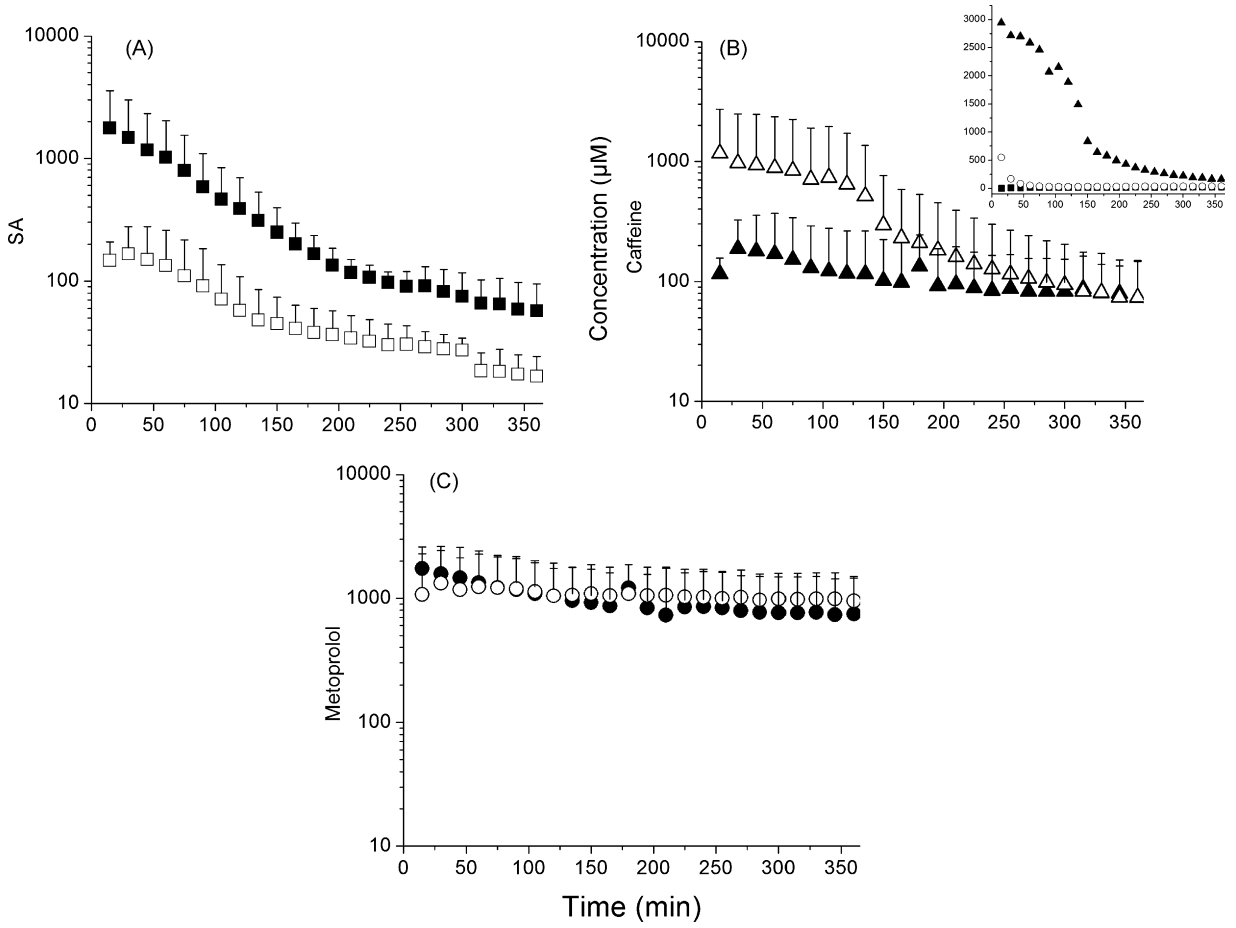

Fig. 6. Comparison of drug concentrations in the ulcer dialysate as a function of size. (A) Small (□) and large (■) ulcers when SA was dosed, (B) small (△) and large (▲) ulcers when caffeine was dosed; inset is the individual submucosal dialysate curves for each rat used to construct the average curve and (C) small (◯) and large (●) ulcers when metoprolol was dosed ($n = 3$ for each curve).

3.6. Absorption as a function of ulcer size

As demonstrated by the UI values, ulcers formed chemical induction of 20% acetic acid injection with the addition of a coating of lubricant over the injection site resulted in ulcers half the size as ulcers formed without lubricant (small versus large ulcers). To determine the effects of ulcer size on absorption, the test compounds were dosed to large ulcer stomach models in addition to the above described small ulcer stomach models. Fig. 6 shows comparison of concentrations observed in the large and small ulcers for each dosed compound. In general, concentrations in the ulcerated submucosa of the larger ulcers were greater than in the small ulcer submucosa. For SA, there was an overall 7-fold increase in concentration in the large ulcer tissue relative to the small ulcer. For caffeine, the average caffeine concentration in the small ulcer was greater than the large ulcer. This appearance of an increase was found to be due to one of the rats exhibiting extremely high concentrations creating a bias in the average toward that one result. The inset plot of Fig. 6B shows the individual results for the small ulcer tissue. As illustrated, one rat exhibits higher concentrations than the remaining two rats. Interestingly, for metoprolol, there was no difference in concentration in the ulcer tissue as a function of size.

4. Discussion

The ability to compare diseased tissue to normal, healthy tissue would give a better understanding to observable differences between these two tissue types. By microdialysis sampling in both healthy and ulcerated tissue of the same stomach, a direct comparison between these tissue types was achieved. This direct comparison resulted in decreased variability due to multiple animal use required for traditional methods.

4.1. Method of chemical ulcer induction

In order to implant a microdialysis probe into the intact ulcerated tissue, the ulcer needed to be site-specific and visible from the serosal side of the stomach. Traditional methods for chemical ulcer induction focus mainly on oral gavage of the ulcer causing agent [17,18]. While successful at producing an ulcer, the ulcer can only be viewed endoscopically in the intact stomach [19,20]. In addition, ulceration would occur across the entire stomach tissue making the direct comparison of ulcerated and healthy tissue difficult. The submucosal injection of 20% acetic acid was found to produce ulcers visible on the serosal side with a controlled area of ulceration [13]. Reported with this method was the frequent perforation of the ulcer tissue due to adherence to the liver and adipose tissue. For the present research, a modification to the procedure of coating lubricant over the ulcer area was found to be effective in the reduction of ulcer perforation and increasing the success rate of the experiment. Although the addition of the lubricant results in a decrease in ulcer size, it is suggested to be a necessary addition to the induction method to increase experimental success.

4.2. Direct comparison of absorption in ulcerated and healthy tissue

Monitoring drug absorption of test compounds through the stomach was performed to determine the significance of a multiple probe approach to directly comparing ulcerated and healthy tissue. SA, caffeine and metoprolol were chosen due to the well defined absorption characteristics through the stomach. More test compounds with differing degrees of absorption or different mechanisms of transport across the stomach would further characterize this approach for drug absorption applications. It was expected that without the mucosa present in the ulcerated region of the stomach, an increased absorption through the ulcer tissue would occur. The concentration-time curves and pharmacokinetics analyses support increased absorption in the ulcer tissue relative to healthy tissue. The use of a multiple probe approach to compare these tissue types in the same stomach allowed for more subtle changes to be assessed as well as obtain a complete time-course from a single animal even further reducing biological variability.

4.3. Considerations for microdialysis sampling in ulcerated tissue

In the present study, variations in concentrations determined in the ulcer were observed. Based on these studies, it is suggested that contributions to variations include ulcer size, ulcer thickness, probe location within the ulcer and proximity of probe to neighboring tissue type. As illustrated in Fig. 6, absorption was found to increase as a function of ulcer size. In conjunction with size, ulcer thickness was found to affect the concentration of drug determined in the ulcerated tissue. A thinner ulcerated submucosa ($\sim$500 μm) generally resulted in increased tissue concentrations relative to thicker ulcerated submucosa ($\sim$1100 μm). This was determined to be the cause of the high concentrations observed in the one rat illustrated in the inset of Fig. 6B. Additionally, the location of the probe within the ulcerated tissue was found to affect the observed drug concentrations in the ulcer tissue. A probe implanted in the ulcerated tissue at a depth closer to the lumen resulted in higher concentrations relative to a probe implanted closer to the serosal surface (*i.e.* more superficial). Another consideration with a multiple probe approach is the proximity of the probe to the differing tissue type. A general concern for microdialysis sampling is the potential cross-talk of probes due to their proximity within a tissue [21]. For these studies, the probes were implanted on the ventral surface of the stomach. With surface area limitations, a probe targeted for sampling in the healthy submucosa that is implanted near ulcerated tissue could have a "tissue cross-talk" effect. This was the possible source of the appearance of metoprolol in the one rat with observable concentrations in the healthy submucosa (Fig. 5). Overall, with these considerations in mind, the use of a multiple probe microdialysis sampling approach offers an unparalleled opportunity to directly compare both ulcerated and healthy tissue in the same stomach.

5. Conclusions

A multiple probe microdialysis sampling approach to sampling in both ulcerated and healthy tissue of the same stomach would serve as a better model to directly compare these different tissue types. For this research, methods of a multi-probe microdialysis approach of implanting probes in the stomach lumen, in both healthy and ulcerated submucosa and in the blood of the same rat were presented. Drug absorption studies through the stomach were performed to examine the significance of this approach that demonstrated increases in concentrations observed in the ulcer tissue relative to healthy tissue. The increased concentrations were found to be a function of ulcer size and thickness and probe location within the tissues.

The research presented focused on the use of a multiple probe approach to compare drug absorption through both the ulcerated and healthy tissue. As a further application, this approach should also be considered to sample both diseased and healthy tissue during disease formation and healing to further study changes in tissues as well as identify and monitor biomarkers for GI disease.

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References

[1] P.O. Gubbins, K.E. Bertch, Clin. Pharmacokinet. 21 (1991) 431–447.
[2] M.J.S. Langman, in: J.G. Hardy, S.S. Davis, C.G. Wilson (Eds.), Drug Delivery to the Gastrointestinal Tract, Ellis Horwood Limited, New York, 1989, pp. 221–228.
[3] S.H. Caldwell, R.W. McCallum, in: M.J. Collen, S.B. Benjamin (Eds.), Pharmacology of Peptic Ulcer Disease, Springer-Verlag, Berlin, 1991, pp. 189–227.
[4] W.G. Thompson, The Ulcer Story: The Authoritative Guide to Ulcers, Dyspepsia, and Heartburn, Plenum Press, New York, 1996.
[5] K. Iwanaga, T. Kushibiki, M. Miyazaki, M. Kakemi, Biol. Pharm. Bull. 29 (2006) 508–512.
[6] M.-H. Shyr, L.-C. Lin, T.-Y. Lin, T.-H. Tsai, Anal. Chim. Acta 558 (2006) 16–21.
[7] Q. Shen, Y. Lin, T. Handa, M. Doi, M. Sugie, K. Wakayama, N. Okada, T. Fujita, A. Yamamoto, Int. J. Pharm. 313 (2006) 49–56.
[8] A. Kaddoumi, D. Fleisher, T. Heimbach, L.Y. Li, S. Cole, J. Pharm. Sci. 95 (2006) 435–445.
[9] J. Domenech, M. Alba, J.M. Morera, R. Obach, J.M. Pla Delfina, Br. J. Clin. Pharmacol. 19 (Suppl. 2) (1985) 85S–89S.
[10] L.S. Schanker, P.A. Shore, B.B. Brodie, C.A. Hogben, J. Pharmacol. Exp. Ther. 120 (1957) 528–539.
[11] J.M. Ault, C.M. Riley, N.M. Meltzer, C.E. Lunte, Pharm. Res. 11 (1994) 1631–1639.
[12] M. Telting-Diaz, D.O. Scott, C.E. Lunte, Anal. Chem. 64 (1992) 806–810.
[13] K. Takagi, S. Okabe, R. Saziki, Jpn. J. Pharmacol. 19 (1969) 418–426.
[14] Y. Song, C.E. Lunte, Anal. Chim. Acta 400 (1999) 143–152.
[15] Y.M. Choi, S.M. Chung, W.L. Chiou, Pharm. Res. 12 (1995) 1323–1327.
[16] A. Yu, L. Shargel, Applied Biopharmaceutics & Pharmacokinetics, McGraw-Hill, New York, 1999.
[17] S.I. Chandranath, S.M. Bastaki, J. Singh, Clin. Exp. Pharmacol. Physiol. 29 (2002) 173–180.
[18] L.P. Singh, P. Kundu, K. Ganguly, A. Mishra, S. Swarnakar, Free Radical Biol. Med. 43 (2007) 289–299.
[19] T. Kamada, J. Hata, H. Kusunoki, K. Sugiu, T. Tanimoto, M. Mihara, H. Hamada, S. Kido, Q. Dongmei, K. Haruma, J. Gastroenterol. Hepatol. 21 (2006) 98–102.
[20] L. Wang, C.P. Hu, P.Y. Deng, S.S. Shen, H.Q. Zhu, J.S. Ding, G.S. Tan, Y.J. Li, Planta Med. 71 (2005) 416–419.
[21] M.I. Davies, C.E. Lunte, Life Sci. 59 (1996) 1001–1013.