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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.

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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

were collected for analysis. The calibration solution was flushed from the microdialysis probes by perfusion with Ringer's or artificial gastric solution. The probes were flushed 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 solution in the stomach was removed by pulling on the syringe connected to the gavage tube. The dose was given by gavage as a bolus dose by connecting a 5 mL syringe containing 5 mM analyte dissolved in artificial gastric solution to the gavage tube. Test compounds were administered in 3 mL of artificial gastric solution through the gavage tube. Sampling started after correcting for probe dead volume. Microdialysis samples were collected for 6 h post-dose from each probe.

2.8. Histology of stomach tissue

After completion of the experiment, the rats were euthanized by isoflurane inhalation overdose. Post mortem, the stomach solution was removed and replaced with 3 mL of a 10% neutral buffered formalin solution. The ligated stomach was removed and placed in approximately 25 mL of 10% neutral buffered formalin. 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, the slides were studied microscopically. The results of the tissue slides were discussed with a pathologist.

2.9. 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 (50 mM; pH 2.5)/acetonitrile (75/25, v/v), flow rate = 0.35 mL/min [16]. 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.

The Food and Drug Administration (FDA) Guidance for Bioanalytical Method Development and a report by Peters et al. [17] were consulted for the acceptance criterion for HPLC–UV method validation. 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.

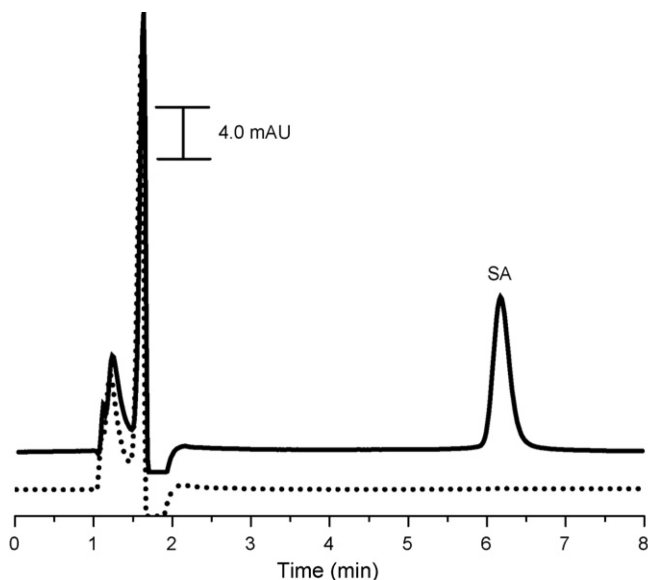

Fig. 1. Mucosa dialysate chromatogram collected prior to dose (dotted line) and 15 min after a 5 mM SA p.o. dose (solid line).

2.10. Data analysis

Concentration–time curves (concentrations corrected for probe extraction efficiency) were generated with Microsoft Excel and OriginLab version 6.0 software (Northampton, MA). Pharmacokinetic parameters were calculated using WinNonlin version 4.1 software (Pharsight, Mountain View, CA). To describe absorption from the lumen, a one-compartment, first-order elimination was used (WinNonlin Model 1) [18,19]. Modeling for the mucosa, submucosa and blood was described by a one compartment, first-order process (WinNonlin Model 3) [19]. The area under the curve (AUC) was determined by the trapezoidal rule. Statistical analysis of the pharmacokinetics was performed with OriginLab software by the one-way ANOVA followed by a Tukey 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 from mucosal dialysate taken prior to and 15 min after a 5 mM oral dose of SA was given by gavage. These chromatograms represent good method selectivity for SA. The same selectivity was observed with all analytes in all probe locations. The calibration curves were linear over the range of 1–200 μM with a goodness of fit of 0.99–1. The intra-assay precision was 97% or greater for all calibration curves. The limits of detection for SA, caffeine and metoprolol were 200, 100 and 200 nM, respectively.

3.2. Stomach tissue layer thickness

To determine the capability of implanting microdialysis probes into the mucosa and submucosa separately, measurements of the stomach layer thickness were taken. Excised stomachs, with no microdialysis probes implanted, were processed for histology as described above. The slides were viewed under a light microscope and layer thickness of the mucosa, submucosa, muscularis

Table 1
Female Sprague–Dawley rat stomach layer thickness

Rat stomach tissue layer	Thickness of layer (mm) ($n = 15$)
Mucosa	0.69 ± 0.28
Submucosa	0.50 ± 0.42
Muscularis externa	0.21 ± 0.21
Entire stomach	1.40 ± 0.49

externa and the entire stomach tissue were measured. Table 1 describes the measured thickness of each layer of the rat stomach. Overall, the mucosa is approximately 35% of the entire tissue thickness with more variation in thickness observed in the submucosa. Based on these results, it was determined that a linear microdialysis probe of a 350 μm outer diameter would be suitable for individual implantation in both the mucosa and submucosa layers.

3.3. Verification of probe implantation in the mucosa and submucosa

Microscopic observation of the stomach tissue histology was performed to verify the location of the probes within the mucosa and submucosa. Fig. 2a shows the probe implantation site in the mucosa of a stomach harvested directly after probe implantation. Fig. 2b shows the probe implantation site in the submucosa of a stomach harvested 2 h after probe implantation. In Fig. 2b, the actual membrane can be seen in the implantation site. Fig. 2c shows a mucosa probe implantation site in a stomach harvested 12 h after probe implantation. These figures represent different time points throughout the experiment. The slides show successful linear microdialysis probe implantation within the individual layers of the stomach. Additionally represented in the slides are minimal perturbation to the tissue upon implantation and no occurrence of a significant immune response for the time course of the experiment.

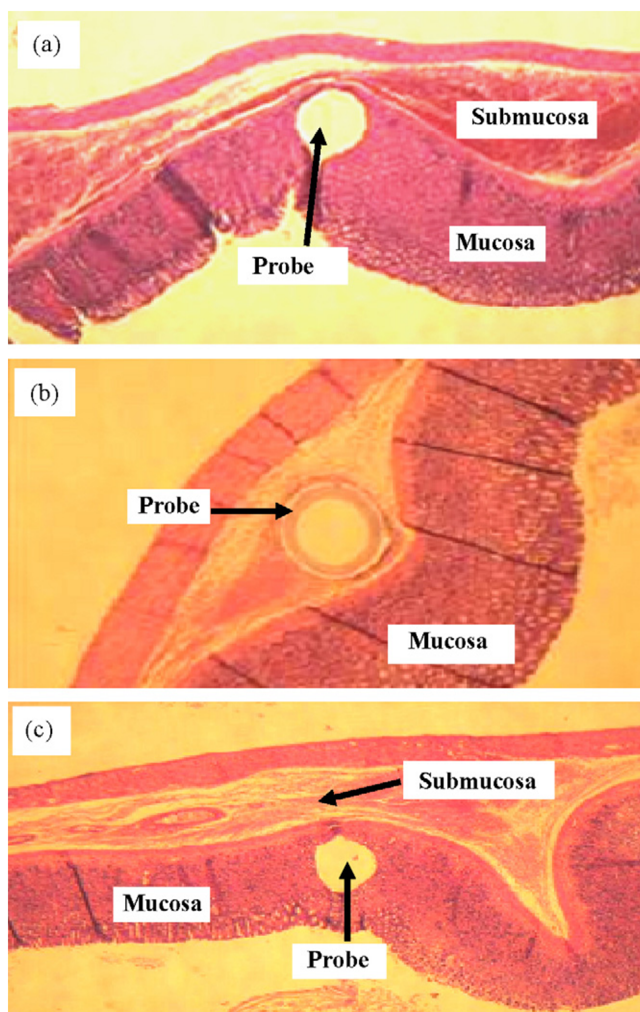

Fig. 2. Histology images of microdialysis probe implantation (a) in the mucosa right after implantation, (b) in the submucosa 2 h post implantation and (c) in the mucosa 12 h post implantation. Images at 20× magnification.

Table 2
Extraction efficiencies determined by analyte delivery (10 μM; 1 μL/min) through the probe

Location of probe	Extraction efficiency (%)		
	Salicylic acid	Caffeine	Metoprolol
Lumen	61.8 ± 6.6 (8)	63.0 ± 11.5 (11)	44.2 ± 9.2 (7)
Mucosa	37.9 ± 13.1 (6)	36.3 ± 6.0 (5)	29.7 ± 8.5 (7)
Submucosa	32.7 ± 12.4 (9)	39.4 ± 13.6 (14)	30.8 ± 6.5 (7)
Blood	52.8 ± 12.6 (19)	54.6 ± 13.0 (15)	32.7 ± 7.1 (6)

Values are average ± standard deviation (n value).

3.4. Microdialysis probe extraction efficiency

The extraction efficiency values from delivery of analyte for each compound studied and for each probe are illustrated in Table 2. Overall, extraction efficiencies between test compounds within each studied region were similar. As expected, higher extraction efficiencies were generally seen in the lumen and blood. Approximately 30% delivery was determined for both the mucosa and submucosa. Calibration of the probes occurred prior to the dosing the analyte. Table 2 shows the collective extraction efficiency values for all probes that were successfully calibrated for each analyte and each probe location for this research. Only experiments with all four functional probes correctly implanted in one animal were used for the drug absorption studies.

3.5. Drug absorption studies from multiple probe microdialysis sampling

3.5.1. Concentration–time curves

Semilog concentration–time curves of microdialysis sampling in the stomach lumen, mucosa submucosa and in the blood after a 5 mM dose of SA, caffeine and metoprolol are shown in Figs. 3–5, respectively. For experiments of dosed SA or caffeine, concentrations of drug were detected in all of the studied sites in the first sample collected (15 min post-dose). The concentrations of SA in the lumen decreased over the course of the experiment, indicating absorption of drug from the lumen. Caffeine luminal concentrations also decreased over time; however, this decrease in caffeine was slower than observed with SA. A steady metoprolol concentration was observed in the lumen throughout the sampling period. The changes in the lumen were concluded to be due to absorption events from the stomach rather than analyte degradation or stomach volume changes. All analytes were found to be stable in artificial gastric solution, which was the primary component in the stomach lumen since the rats were fasted prior to experimentation. Prior to dosing and at the end of experimentation, the gastric solution was removed and replaced with dose or formalin solution, respectively. With both removals, approximately 3 mL was recovered indicating no significant volume change over the course of the experiment.

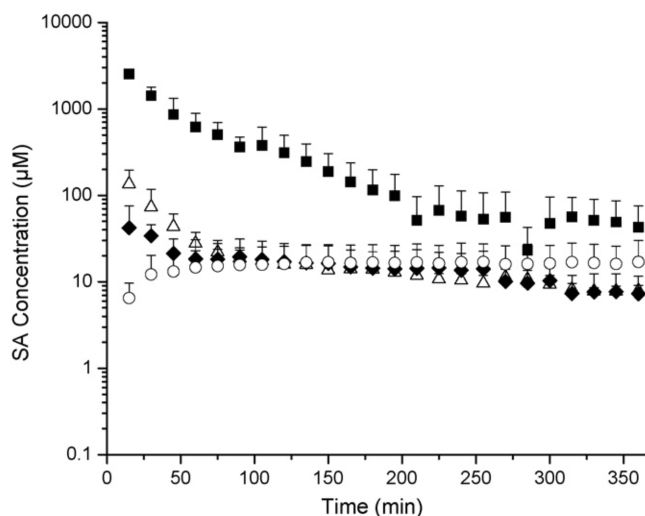

Fig. 3. SA by microdialysis sampling in the lumen (■), mucosa (△), submucosa (♦) and in blood (○) after a 5 mM SA p.o. dose ($n = 4$).

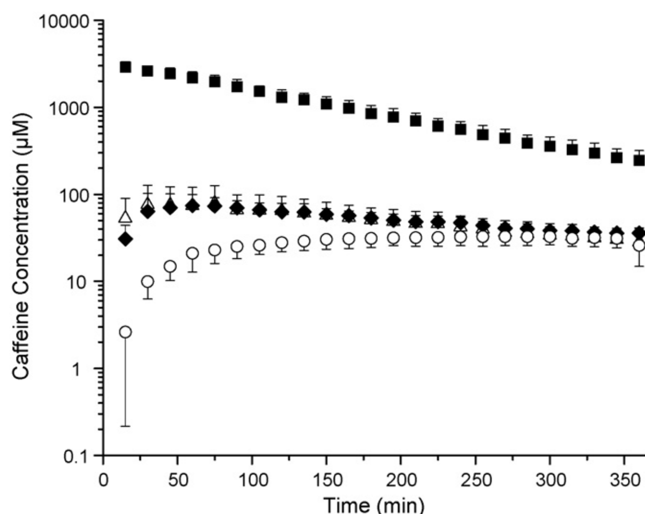

Fig. 4. Caffeine by microdialysis sampling in the lumen (■), mucosa (△), submucosa (♦) and in blood (○) after a 5 mM caffeine p.o. dose ($n = 4$).

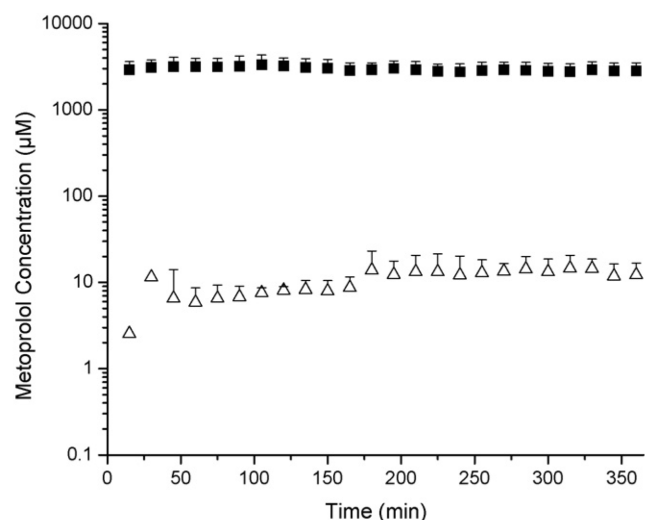

Fig. 5. Metoprolol by microdialysis sampling in the lumen (■) and mucosa (△) after a 5 mM metoprolol p.o. dose ($n = 4$).

Table 3
Pharmacokinetics parameters from the lumen, mucosa, submucosa and blood after a 5 mM bolus p.o. dose for each studied compound ($n = 4$)

	k_e (h^{-1})	C_{max} (µM)	AUC_{0-6h} (µM h)
Lumen[a]			
SA	0.95 ± 0.38[b]	3853 ± 670	2227 ± 460[b]
Caffeine	0.45 ± 0.054[b]	3335 ± 518	7499 ± 1400[b]
Metoprolol	0.024 ± 0.018[b]	3196 ± 832	93043 ± 859[b]
Mucosa			
SA	0.19 ± 0.09[c]	142 ± 57[c,d]	125 ± 24[c,e]
Caffeine	0.20 ± 0.17	83 ± 43	656 ± 301[c]
Metoprolol	0.033 ± 0.018[e]	14 ± 7[e]	497 ± 110
Submucosa			
SA	0.114 ± 0.054[c]	48 ± 30	88 ± 25[c]
Caffeine	0.156 ± 0.042[c]	78 ± 30[c]	460 ± 117[c,e]
Metoprolol	–	–	–
Blood			
SA	0.0024 ± 0.0018[e]	20 ± 12	6637 ± 4864
Caffeine	0.042 ± 0.003	33 ± 7	1102 ± 851
Metoprolol	–	–	–

Values are average ± standard deviation. Statistical analysis by one-way ANOVA followed by a Tukey test. –: No analysis was done; concentrations not detected in these sites.
[a] All parameters significantly different from other sites and for all drugs ($p < 0.05$).
[b] Significantly different across all drugs ($p < 0.05$).
[c] Significantly different from the blood ($p < 0.05$).
[d] Significantly different from the submucosa ($p < 0.05$).
[e] Significantly different relative to other drugs ($p < 0.05$).

Consistently, the concentrations in the mucosa were higher than observed in the submucosa. An absorption phase was detected in the mucosa and submucosa when caffeine was dosed; however, this absorption phase was not seen when SA was dosed. For metoprolol, around 10–15 µM was observed in the mucosa while no metoprolol was detected in the dialysate from either the submucosa or the blood. SA and caffeine concentration profiles in the blood were observed to be similar.

3.5.2. Pharmacokinetics analysis

To numerically characterize the curves from Figs. 3–5, pharmacokinetics parameters were generated for each studied site. Table 3 shows the results from modeling each drug for the lumen, mucosa, submucosa and blood. No modeling was done with metoprolol in the submucosa or the blood since concentrations were below the detection limits of the analytical system.

To assess the overall rates of absorption, the lumen elimination rate constants (k_e) and overall AUC values were compared for each compound. The rate constant for elimination from the lumen (k_e) was faster for SA, then caffeine, and the slowest rate for metoprolol. The AUC in the lumen increased from SA, then caffeine, and to metoprolol as the highest. Additionally noted was no statistical difference in luminal C_{max} values as the same 5 mM bolus dose was given for each compound in the study.

No statistical difference was observed in the elimination rate constants observed between the mucosa and submucosa for both SA and caffeine ($k_e = 0.11–0.20\,h^{-1}$). However, differences were observed in the C_{max} values between the mucosa and submucosa. This difference is particularly evident when metoprolol was dosed since metoprolol was below detectable limits in the submucosa. A threefold increase was observed in the mucosa C_{max} relative to the submucosa when SA was dosed. Also noted were differences in generated parameters between the stomach tissue layers and the blood. The rate of elimination was slow for both SA and caffeine in the blood ($k_e = 0.0024$ and $0.042\,h^{-1}$, respectively) with additional smaller C_{max} values relative to the stomach tissue.

4. Discussion

The rat stomach is a heterogeneous tissue comprised of mainly a mucosa and a submucosa layer. Based on the differences between the tissue layers (*e.g.* tissue type and blood flow to each layer), it was predicted that differences in tissue concentrations would be observable, as demonstrated in the skin, another heterogeneous tissue [20]. With the previous designs of microdialysis sampling in the stomach, only the submucosa was sampled. The present research would serve as an enhancement for gastric microdialysis sampling by allowing for the simultaneous sampling of both the mucosa and submucosa layers.

4.1. Histological examination

Based on the results presented, microdialysis probes can successfully be simultaneously implanted in the stomach lumen, mucosa and submucosa and in the blood of the rat. The histology results confirmed that the linear probes used for this study were of appropriate diameter to be implanted into the individual tissue layers simultaneously. The histology results also showed that over the 12-h experiment, no significant immune response was observed and therefore, no tissue recovery period is required for the current study design. It is therefore suggested that a 1-h "tissue equilibration" time after probe implantation would be sufficient for vasodilation to return to normal to start sampling, as previously determined for other tissues [21]. The use of microdialysis sampling for longer experiments would require an assessment of tissue response to probe implantation in the mucosa and submucosa tissue. Studies by Ericsson et al. showed an inflammatory cell peak around 3 days after implantation in the rat stomach submucosa indicating a 3-day recovery before sampling [7].

4.2. Extraction efficiency examination

The use of 5 mm microdialysis probes in the stomach resulted in a 24% decrease in extraction efficiency versus a 10 mm microdialysis probe (data not shown); however, resulted in greater implantation success over a 10 mm probe. Maintaining a shorter membrane length within the tissue layer was more successful since the implantation method presented relies on the tunneling of the introducer through the tissue layer. Therefore, a 5 mm membrane length was chosen for this research to maximize both probe recovery and probe implantation success.

Similar extraction efficiency values were determined in the mucosa and submucosa. This similarity was not predicted based on the differing tissue types further suggesting that transport across the probe membrane is the rate-limiting step in the current probe design and implantation. Higher values in lumen and blood were expected based on the more hydrodynamic environment of probe location. Unexpected higher extraction efficiency values were determined in the lumen even though the membrane length is half that used for blood sampling. Besides the difference in membrane length between the two types of probes, these probes were perfused with different solutions, Ringer's and artificial gastric solution. In addition, the probes were placed in different pH environments, highly acidic stomach lumen versus blood. Further studies will be needed to investigate if the different perfusion fluid or probe environment is a factor in probe recovery for these studies.

The resulting variability in extraction efficiency values is suggested to be due to probe implantation rather than other potential factors such as probe differences and animal variation. Despite the fact the probes were made in-house, they were fabricated under the same protocol and by the same personnel. Implantation of linear microdialysis probes in tissue layers with 500–700 μm thickness is dependent on surgical technique. Studies using SA as the analyte were conducted first, followed by caffeine and metoprolol studies were conducted last. As shown in Table 2, both the variation and n values decrease from SA to metoprolol suggesting surgical proficiency over the course of this research. Overall, the variability between extraction efficiency accentuates the need to calibrate every implanted microdialysis probe for analyte quantitation.

4.3. Drug absorption from multiple probe microdialysis sampling

Monitoring drug absorption of test compounds through the stomach was performed to determine the significance of a multiple probe approach to gastric microdialysis sampling. The concentration–time curves and pharmacokinetics analysis support the use of a multiple probe microdialysis approach over a single probe approach. Overall absorption rates determined from the lumen probe results were in support of the expected absorption trend from the stomach (SA > caffeine > metoprolol).

Due to the mucosa having a denser tissue type, decreased blood flow and closer proximity to the lumen relative to the submucosa, it was expected that higher concentrations would be observable in the mucosa. The results were in support of these higher concentrations in the mucosa, especially noted when metoprolol was dosed. These results strengthen the use of microdialysis sampling in the separate layers to characterize concentrations in the stomach tissue. Also noted were differences in stomach tissue concentrations relative to the blood. Concentration profiles and pharmacokinetics parameters in the blood were consistently lower than observed in the stomach, giving further rise to the importance of site-specific sampling over traditional blood sampling for pharmacokinetics studies.

5. Conclusions

Due to differences in the mucosa and submucosa layers of the stomach, a multiple probe approach would be more accurate for microdialysis sampling in the stomach. For this research, methods to achieve a multiple probe approach were presented with probes implanted in the stomach lumen, mucosa, submucosa and in the blood of a rat. Studies of drug absorption across the stomach were in support of a multiple probe approach to microdialysis sampling. For GI drug absorption applications, the current results are in support of microdialysis sampling for more accurate site-specific sampling in comparison to traditional methods that either sample only blood or luminal contents. In addition to drug absorption studies, this multiple probe approach should be considered to enhance current uses of gastric microdialysis sampling for monitoring analyte release from cells of the mucosa.

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References

[1] E. Bostrom, U.S. Simonsson, M. Hammarlund-Udenaes, Drug Metab. Dispos. 34 (2006) 1624–1631.
[2] D. Groenendaal, M.C. Blom-Roosemalen, M. Danhof, E.C. Lange, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 822 (2005) 230–237.
[3] F.X. Mathy, D. Ntivunwa, R.K. Verbeeck, V. Preat, J. Pharm. Sci. 94 (2005) 770–780.
[4] Y.T. Wu, T.R. Tsai, L.C. Lin, T.H. Tsai, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 853 (2007) 281–286.
[5] M.I. Davies, C.E. Lunte, Life Sci. 59 (1996) 1001–1013.

[6] M. Bernsand, R. Hakanson, P. Norlen, Br. J. Pharmacol. 152 (2007) 240–248.
[7] P. Ericsson, P. Norlen, M. Bernsand, P. Alm, P. Hoglund, R. Hakanson, Pharmacol. Toxicol. 93 (2003) 57–65.
[8] V. Fykse, E. Solligard, M.O. Bendheim, D. Chen, J.E. Gronbech, A.K. Sandvik, H.L. Waldum, Acta Physiol. 186 (2006) 37–43.
[9] M. Kitano, M. Bernsand, Y. Kishimoto, P. Norlen, R. Hakanson, Y. Haenuki, M. Kudo, J. Hasegawa, Am. J. Physiol. 288 (2005) G1084–G1090.
[10] M. Kitano, P. Norlen, R. Hakanson, Regul. Pept. 86 (2000) 113–123.
[11] J. Domenech, M. Alba, J.M. Morera, R. Obach, J.M. Pla Delfina, Br. J. Clin. Pharmacol. 19 (Suppl. 2) (1985) 85S–89S.
[12] L.S. Schanker, P.A. Shore, B.B. Brodie, C.A. Hogben, J. Pharmacol. Exp. Ther. 120 (1957) 528–539.
[13] J.M. Ault, C.M. Riley, N.M. Meltzer, C.E. Lunte, Pharm. Res. 11 (1994) 1631–1639.
[14] M. Telting-Diaz, D.O. Scott, C.E. Lunte, Anal. Chem. 64 (1992) 806–810.
[15] Y. Song, C.E. Lunte, Anal. Chim. Acta 400 (1999) 143–152.
[16] K.L. Steele, D.O. Scott, C.E. Lunte, Anal. Chim. Acta 246 (1991) 181–186.
[17] F.T. Peters, O.H. Drummer, F. Musshoff, Forensic Sci. Int. 165 (2007) 216–224.
[18] Y.M. Choi, S.M. Chung, W.L. Chiou, Pharm. Res. 12 (1995) 1323–1327.
[19] A. Yu, L. Shargel, Applied Biopharmaceutics & Pharmacokinetics, McGraw-Hill, New York, 1999.
[20] F.X. Mathy, C. Lombry, R.K. Verbeeck, V. Preat, J. Pharm. Sci. 94 (2005) 144–152.
[21] C. Anderson, T. Anderson, K. Wardell, J. Invest. Dermatol. 102 (1994) 807–811.