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RESEARCH ARTICLE

Permeation studies on freshly excised rat gastric mucosa: influence of pH

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Abstract

The objective of this study was to evaluate the influence of pH on the permeation of model drugs through freshly excised rat stomach. Additionally, the capability of excised gastric mucosa to maintain an acidic pH was assessed. *In vitro* permeation studies were performed in Ussing-type diffusion chambers with rat stomach using fluorescence-labeled bacitracin (bac-FITC), sodium fluorescein (NaFlu), propranolol HCl, and cimetidine as model drugs. The pH was adjusted to pH 1, 2, and 6.8 in the donor chamber and pH 7.4 in the acceptor chamber. The study demonstrated that both, the fore stomach and the glandular gastric mucosa, are capable of maintaining an acidic pH of 1–1.2 in the donor chamber. P_{app} (permeation coefficients) were found to be $1.4 \pm 0.6 \times 10^{-7}$ and $7.6 \pm 0.7 \times 10^{-7}$ for bac-FITC and $3.3 \pm 1.5 \times 10^{-7}$ and $2.4 \pm 0.6 \times 10^{-6}$ cm/sec for NaFlu at pH 2 and 6.8, respectively, in the glandular stomach. In order to evaluate the effect of pH on the integrity of paracellular space, propranolol as high-permeability drug and cimetidine as low-permeability drug were chosen. The P_{app} of propranolol HCl was determined to be $5.9 \pm 0.3 \times 10^{-7}$ and $1.1 \pm 0.7 \times 10^{-6}$ cm/sec at pH 2 and 6.8, respectively, in the glandular stomach. Cimetidine showed a permeability of $1.4 \pm 0.4 \times 10^{-5}$ and $9.6 \pm 2.3 \times 10^{-6}$ cm/sec at pH 2 and 6.8. Results provide essential basic information for the development of gastric drug delivery systems.

Keywords: Gastric absorption; Ussing chamber; permeability; pH dependence; cell viability

Introduction

Most of the permeability studies are performed with small intestinal mucosa to evaluate gastrointestinal permeability, although the stomach is capable of absorbing in particular nonionized, lipophilic molecules of moderate size (DeSesso and Jacobson, 2001). Therefore, the gastric mucosa is also of interest for the systemic uptake of various drugs. Stomach-specific delivery of peptides and proteins, for example, would exclude intestinal proteases as a barrier for systemic drug uptake. Hence, only gastric digestive enzymes need to be inhibited by pepstatin or similar inhibitors. Another limitation of drug absorption is represented by P-glycoprotein (P-gp) efflux pumps in many tissues. P-gp is widely expressed, for example on the membranes of endothelial cells in the intestine (Dahan and Amidon, 2009). However, Thiebaut et al. (1987) could not detect P-gp in human stomach tissue. Regarding this aspect, the stomach gains importance as an absorption organ. For example, anticancer drugs, anti-HIV protease inhibitors, antiarrhythmics, and antibiotics have been reported to be efflux pump substrates (Varma et al., 2003). Apart from therapeutic peptides and P-gp substrates, there are also various other drugs for which gastric uptake might be beneficial. For instance, there are several prominent examples of drugs: riboflavin (Akiyama et al., 1998) and furosemide (Menon et al., 1991), which have their absorption window in the stomach. Pharmacokinetic studies of α-lipoic acid suggest the stomach as main absorption organ for this drug due to rapid uptake after oral administration (Bernkop-Schnürch et al., 2004). Furthermore, other drugs like diazepam, chlordiazepoxide, and

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verapamil exhibit a low solubility at high pH values, or are unstable in the intestinal or colonic environment (captopril) (Streubel et al., 2006). Natural antioxidants such as flavonoids are assumed to contribute to the prevention of cardiovascular diseases. Some flavonoids such as quercetin, daizein, or anthocyanins are known to be absorbed as their aglycons from the gastric mucosa (Piskula et al., 1999; Crespy et al., 2002; Passamonti et al., 2003). Although the stomach represents the absorption window for various classes of orally administered drugs, to our knowledge, there is so far no *in vitro* test system for gastric drug permeation studies available.

It was therefore the aim of this study to provide an insight into *in vitro* permeation studies with gastric mucosa, focusing especially on the influence of pH on drug uptake. Ussing-type chambers utilized with rat gastric mucosa were used for investigations. In this respect, it was the goal to determine the capacity of the gastric mucosa to maintain an acidic pH *in vitro*, which indicates the activity of the physiological proton pumps. The activity of the proton pump was demonstrated with pH measurements at specific inactivating conditions, using 0.2 mM omeprazole and an environment of 4°C. To demonstrate the pH-dependent lipophilicity of the used model compounds, the log*D* (distribution coefficient) values were determined at pH 2 and 6.8. Cell viability was controlled throughout all studies via histological examinations.

Materials and methods

Materials

Sodium fluorescein (NaFlu) was purchased from FLUKA (Buchs, Switzerland). Fluorescein-5(6)-isothiocyanate (FITC), bacitracin, cimetidine, propranolol hydrochloride, omeprazole and *N*-(2-hydroxyethyl)-piperazin-*N*'-(2-ethanesulfonic acid) (HEPES) were obtained from Sigma (Vienna, Austria). *N*-Octanol was purchased from ACROS (Geel, Belgium). Sodium chloride, magnesium sulfate, potassium chloride, glucose, sodium bicarbonate were obtained from GATT KOLLER (Absam, Austria).

Methods

FITC labeling of bacitracin

Bacitracin was chosen as model drug because of its stability toward an enzymatic degradation caused by membrane bound peptidases. First, 2 mg of FITC dissolved in 1 mL of dimethyl sulfoxide (DMSO) were gradually added in aliquot volumes of 25 µL to 40 mg of bacitracin dissolved in 20 mL of 0.1 M Na_2CO_3. The coupling reaction was stopped after 8 h of incubation at 4°C by adding NH_4Cl to a final concentration of 50 mM. The resulting bacitratin–FITC (bac-FITC) conjugate was incubated for 2 h at 4°C, isolated by gel filtration (Sephadex G15, Amersham Biosciences Europe, Vienna, Austria), and lyophilized at –70°C and 0.01 mbar (Benchtop 2K, VirTis, NY).

Histological investigations

For histological investigations, freshly excised gastric tissue was incubated for 3 h in Ussing-type chambers. Studies were carried out at pH 1 for the apical (AP) side and pH 7.4 for the basolateral (BL) side of the gastric mucosa, followed by incubation with trypan blue solution 0.4% (w/v) for 20 min. After washing with a buffer of pH 7.4, the samples were fixed with Bouins solution (Romeis, 1968), embedded in paraffin, and cut into 7-µm thick slices. The paraffin was removed with xylol and the slices were covered with Entellan® for conservation. The procedure was also repeated with freshly excised gastric tissue without incubation at acidic pH.

pH Measurements

This investigation used the gastric mucosa of non-fasted rats (Sprague-Dawley, male, weighing 250–350 g), dividing the stomach into the glandular stomach and fore stomach tissue. In order to evaluate the capability of the gastric mucosa to maintain an acidic pH, freshly excised gastric tissue was mounted in Ussing-type chambers (0.64 cm^2 surface area). The donor chamber was filled with 1 mL 80 mM HCl/0.2% NaCl (U.S. Pharmacopeia, 30) adjusted to pH 1 and 2, whereas 1 mL incubation medium pH 7.4 containing 250 mM NaCl, 2.6 mM $MgSO_4$, 10 mM KCl, 40 mM glucose, and 50 mM $NaHCO_3$, buffered with 40 mM HEPES, was added to the acceptor chamber. The pH was determined using a pH meter (SenTix Mic, WTW, Weinheim) in 5 min intervals for 30 min, and in 30 min intervals for 120 and 180 min. All studies were performed at 37°C and 4°C with and without 0.2 mM omeprazole in the donor chamber.

In vitro permeation studies

In vitro permeation was carried out using non-fasted rats (Sprague-Dawley, male, weighing 250–350 g), in which the stomach and small intestine (20 cm) were immediately removed after sacrificing. The stomach was further divided into the glandular stomach and fore stomach tissue. Stomach contents were carefully removed without destroying the mucus layer. Afterward, the tissue was cut into pieces of 1.5 × 1.5 cm and mounted in Ussing-type chambers (0.64 cm^2 surface area), avoiding stripping off the underlying muscle layer. Rat small intestine was also carefully washed, cut into strips of 1.5–2 cm, and mounted in Ussing-type chambers, avoiding stripping off the underlying muscle layer. Preheated (37°C) incubation medium as described earlier, adjusted to pH 6.8 or 7.4, was added to both sides (AP and BL) of the chambers (1 mL) mimicking intestinal fluid. Alternatively, to simulate gastric conditions, 1.0 mL of 80 mM HCl/0.2% NaCl (U.S. Pharmacopeia, 30) adjusted to pH 2 was added to the donor chamber. Before beginning the permeability measurements, a 30-min equilibration period was allowed. For absorption studies, NaFlu and bac-FITC in a final concentration of 0.001% (w/v) and 0.1% (w/v), respectively, were added to the donor compartment. Propranolol HCl (200 µmol/L) and cimetidine (2 mM)

were added as model compounds to the donor chamber. As NaFlu is poorly soluble at pH 2, 2% (v/v) of DMSO was added as solvent to the donor chamber. Referring to Da Violante et al. (2002), DMSO does not exhibit cytotoxicity or permeability-enhancing properties when being used in this concentration. The permeation experiment was carried out over a time period of 150 and 180 min. One hundred microliters of samples were withdrawn from the acceptor compartments at set time points every 30 min. Each time, the removed quantity was replaced by fresh artificial intestinal fluid equilibrated at 37°C. All experiments were performed in an atmosphere of 95% O_2 and 5% CO_2 at 37°C.

The amount of permeated markers was measured using a microplate reader at a wavelength of 480 nm for excitation and 520 nm for emission (Fluostar Galaxy, BMG, Austria). Cumulative corrections were made for previously removed samples. The permeability of propranolol HCl and cimetidine were determined by HPLC analysis.

HPLC Analysis

Samples analysis was performed via reversed-phase HPLC using a Hitachi LaChrome Elite series L2130 pump, Hitachi LaChrome Elite series L-2200 autosampler, and a Hitachi LaChrome Elite L-2450 diode array detector.

Propranolol was quantified by an HPLC method described by Panchagnula et al. and cimetidine by an HPLC method described by Iqbal and others (Iqbal et al., 2004; Panchagnula et al., 2004). For both analyses, a LiChrospher® 100 RP18 (5 μm) 125 × 4 mm column was used.

Data analysis

Apparent permeability coefficients for NaFlu and bac-FITC were calculated according to the equation $P_{app} = Q/(A \times c \times t)$, where P_{app} is the apparent permeability coefficient (cm/sec), Q is the total amount of test substance permeated through the mucosa (μg), A is the diffusion area of the Ussing-type chamber (cm^2), c is the initial concentration of the marker substance in the donor compartment ($\mu g/cm^3$), and t is the total time of the experiment (sec).

logD determination

NaFlu and bac-FITC were dissolved in final concentrations of 0.001% and 0.1%, respectively, in 1.0 mL of medium used in permeation studies. To each of these solutions, 1.0 mL of *n*-octanol having been saturated with the corresponding buffer solutions were added. Samples were shaken at 1300 rpm (Eppendorf Thermomixer comfort) at 25°C ± 0.2°C for 1 h. The aqueous phase was withdrawn and aliquots of 0.5 mL were centrifuged (VWR Galaxy 7D) for 5 min at 13,000 rpm at room temperature. The concentration of each fluorescence marker was quantified as described earlier. As control, the samples without any test compound were analyzed as well. In addition, the concentration of test compound in the samples before *n*-octanol was added has been determined.

Statistical data analyses

Statistical data analysis was performed using the Student's *t*-test with $P < 0.05$ as the minimal level of significance unless indicated otherwise.

Results and discussion

Histological investigations

From the histological point of view, the gastric mucosa of the rat exhibits several differences to the human one. Both humans and rats have a single-chambered stomach, but the stomach of the rat exhibits two distinctly different, grossly discernible regions (DeSesso and Jacobson, 2001). The anatomy of rat stomach is illustrated in Figure 1. The fore stomach, which is keratinized (Luciano and Reale, 1992), is an entry from the esophagus, while the mucosa of the glandular stomach simulated approximately the human conditions and seems to be much more valid for permeation studies. The influence of the pH on the absorption ability in the stomach is comparable with the strong influence of the lipophilicity, which is pH-dependent for ionizable drugs.

In order to evaluate the effect of pH via *in vitro* permeation studies, the viability of gastric mucosa at low and almost neutral pH values was investigated. Results demonstrated that freshly excised gastric mucosa is not harmed within 3 h when being exposed to pH 1–2 at the AP side of the tissue. Cell viability was confirmed by staining with trypan blue and additional histological examinations after 3 h of incubation. In Figure 2, the histological appearance of gastric mucosa before and after acidic exposure is shown. During this experiment, the AP membrane was exposed to a proton gradient of a maximum around 106. Although the AP membrane transports bicarbonate to protect toward acidic attack, it has been demonstrated that at low luminal pH this gradient is rapidly dissipated, leaving the AP membrane itself exposed to secreted acid (Takeuchi et al., 1983).

The simple observation that gastric mucosa is able to maintain its morphologic integrity during acid exposure allows permeation studies at low pH, without damaging the cell structure. The reactivity of trypan blue, a

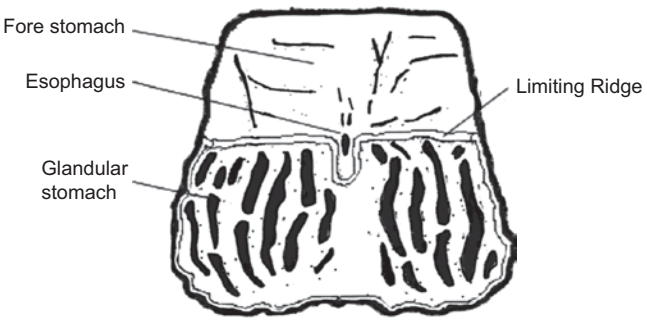

Figure 1. Schematic presentation of the anatomy of rat stomach; fore and glandular stomach are divided by the limiting ridge; fore stomach is keratinized; glandular stomach contains mucus and glandular tissue.

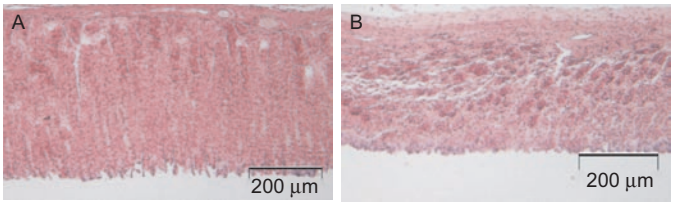

Figure 2. Histological appearance of gastric mucosa before (A) and after (B) acidic exposure over a period of 3 h.

vital dye, is based on the fact that the chromophore is negatively charged and does not interact with the cell unless the membrane is damaged. Therefore, all cells that exclude the dye are viable (Freshney, 2005). As shown in Figure 2B, the number of blue-colored cells does not increase significantly compared with that in Figure 2A.

pH Measurements

For *in vitro* permeation, it was investigated whether freshly excised gastric mucosa can maintain a pH gradient of 1–2 on the AP side and a pH of 7–8 on the BL side. A pH of 7.4 on the serosal side of the tissue was chosen to mimic physiological environment and in order not to harm the tissue, as it is well-known that the serosal side of the gastric tissue is much more susceptible to acidification (Ashley et al., 1987). Results demonstrated that freshly excised glandular stomach maintains this pH gradient, when the pH on the AP sides is adjusted to 1. In contrast, when adjusted to pH 2, the pH increased from 2 to 6.1 in the donor chamber within 3 h. The pH in the acceptor chamber of the glandular stomach decreased from pH 7.4 to 7.3, when the pH in the donor chamber has been adjusted to pH 1. In contrast, when the pH in the donor chamber has been adjusted to pH 2, the pH on the BL side increased slightly from pH 7.4 to 8.0 within 3 h. Results of this study are shown in Figure 3. In brief, the AP membrane at the glandular stomach maintained the pH constant, when an initial solution of pH 1 was used, whereas a decrease of the protons concentration occurred, when the initial pH was 2. An explanation for the observed phenomenon is most likely the low initial buffer capacity of HCl solution at pH 2. On the serosal side the pH started to decrease after 120 min, likely because of already reduced poor viability of the tissue. Despite the results of the histological investigations, the combination of factors, like the acidic milieu and the mechanical stress by the pH microelectrode, might impair the viability of the stomach. The slight increase in pH in the acceptor chamber, starting at pH 2, could be probably caused by the addition of 50 mM $NaHCO_3$ and consequently this effected a decrease of CO_2 during the experiment.

Proton pump activity was disabled using 0.2 mM omeprazole and an environment of 4°C. At 4°C, ATP-dependent mechanisms are quenched. Consequently, proton pumps also are inactive at this temperature. The results confirming this statement are shown at Figure 4. The pH in the donor chamber increased significantly from 1.0 to 1.3 ± 0.01 at 4°C compared with 37°C after 5 min. After 30 min, the pH increased at 4°C and in

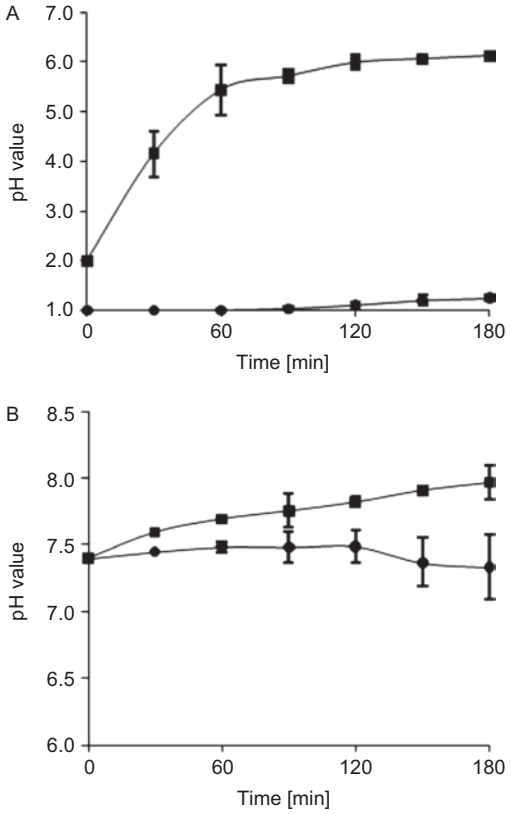

Figure 3. The pH profile of the rat glandular stomach mucosa; pH profile in the donor chamber (A) starting from pH 1 (1.0 mL of 80 mM HCl/0.2% NaCl) (•) and pH 2 (1.0 mL of 80 mM HCl/0.2% NaCl adjusted to pH 2) (■); pH profile in the acceptor chamber (B) starting from pH 7.4 (HEPES buffer) in both cases. All studies were performed at 37°C. Indicated values are means of three experiments (mean ± SD, $n = 3$).

presence of 0.2 mM omeprazole to around 1.5, whereas at 37°C the pH was determined to be 1.1. In the acceptor chamber, the pH remained at pH 7–8 over an observation period of 120 min. An overview on these results is provided in Table 1. This outcome demonstrated that the inactivation of the proton pumps was obtained at 4°C immediately and in presence of 0.2 mM omeprazole after 30 min. This observation could be presumed by the fact that substituted benzimidazoles require an acid-induced activation (Kromer et al., 1998). Our study proved that the proton pumps are still active and seem to be capable of maintaining an almost constant pH gradient over the 3-h observation period (Table 1). Therefore, it seems reasonable to carry out *in vitro* permeation studies at an acidic milieu. Based on these studies, absorption studies

of weakly acidic drugs through gastric mucosa could be of interest since it is known that the absorption of weak acids will increase at low pH (Karlarli, 1989).

In vitro permeation studies and log*D* determination

In order to evaluate the influence of pH on gastric absorption, the uptake of NaFlu and bac-FITC was evaluated at an initial pH of 2 and 6.8 in the donor compartment. Results of this study are shown in Figures 5 and 6. In addition, obtained P_{app} values are listed in Table 2. Generally, there was a trend showing that the uptake of NaFlu and bac-FITC from the glandular stomach is higher at the high pH level. According to this observation, the oral bioavailability of hydrophilic drugs being absorbed from the gastric mucosa should be significantly higher at time periods when the acidic secretion of the stomach is low. These findings are in good agreement with results obtained in vagotomized dogs, which mimic a low gastric acidity in humans. In vagotomized dogs, the total area under the plasma concentration–time curve for amlodipine from time 0 to the last measured time 48 h in plasma ($AUC_{0-48\ h}$) was significantly greater (725 versus 348 ng h/mL) than those in dogs without vagotomy (Kwak et al., 2006). Since amlodipine is a weak basic drug with pK_a of 8.7, this effect might be related to a major unionized fraction of amlodipine existing in less acidic conditions.

Further permeation studies were performed with propranolol HCl, a high-permeability drug, and

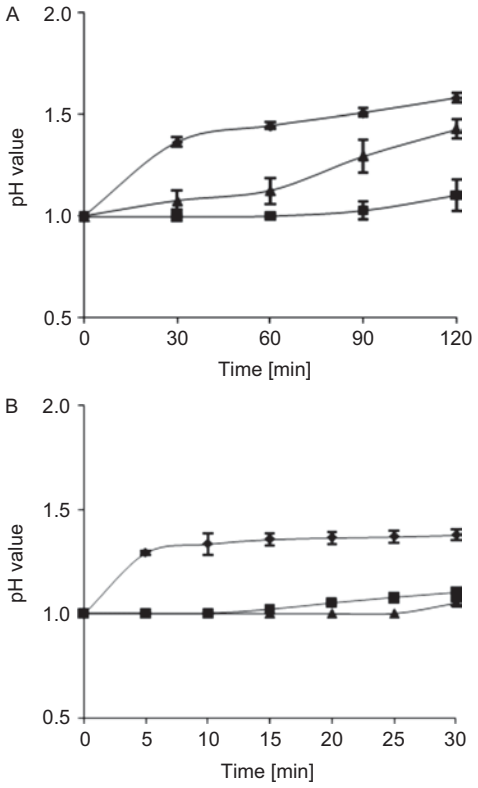

Figure 4. The pH profile of glandular stomach starting from pH 1 (1.0 mL of 80 mM HCl/0.2% NaCl) at 37°C (■), 4°C (♦), and in the presence of the proton pump inhibitor omeprazole (0.2 mM) (▲); (A) observation over a period of 120 min, (B) over a period of 30 min. Indicated values are means of three experiments (mean ± SD; $n = 3$).

Table 1. Results of the pH measurements from the glandular stomach in the donor chamber at 4°C, at 37°C and in presence of 0.2 mM omeprazole, the degree of ionization was calculated.

Conditions	Time (min)	pH value ± SD	Ionic strength [H_3O^+]
37°C	5	1.00 ± 0.00	0.10 M
4°C	5	1.29 ± 0.01	0.05 M
0.2 mM omeprazole	5	1.00 ± 0.00	0.10 M
37°C	30	1.00 ± 0.00	0.08 M
4°C	30	1.38 ± 0.03	0.04 M
0.2 mM omeprazole	30	1.05 ± 0.01	0.09 M
37°C	120	1.10 ± 0.08	0.08 M
4°C	120	1.58 ± 0.02	0.02 M
0.2 mM omeprazole	120	1.43 ± 0.05	0.03 M

Indicated values are means of three experiments (mean ± SD; $n = 3$).

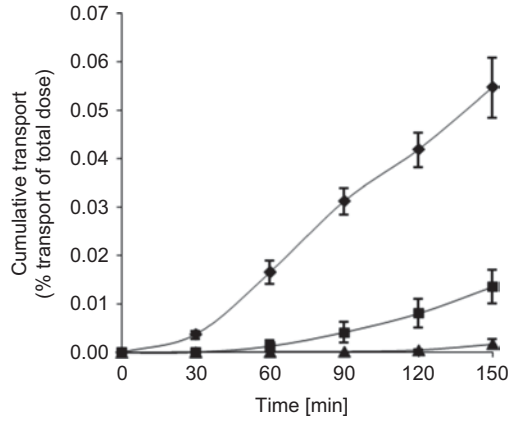

Figure 5. Transport of 0.001% sodium fluorescein/2% DMSO; small intestinal mucosa pH 6.8 (HEPES buffer) (♦), glandular stomach pH 6.8 (■), glandular stomach pH 2 (▲). All experiments were performed in an atmosphere of 95% O_2 and 5% CO_2 at 37°C. Indicated values are means of three experiments (mean ± SD; $n = 3$).

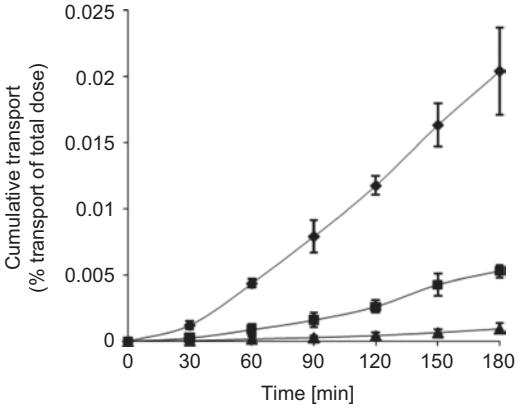

Figure 6. Transport of 0.1% bacitracin-FITC; small intestinal mucosa pH 6.8 (♦), glandular stomach pH 6.8 (■), glandular stomach pH 2 (▲). All experiments were performed in an atmosphere of 95% O_2 and 5% CO_2 at 37°C. Indicated values are means of three experiments (mean ± SD, $n = 3$).

Table 2. Comparison of the influence of pH on the apparent permeability coefficient (P_{app}) of sodium fluorescein, bacitracin-FITC, propranolol HCl, and cimetidine across gastric and intestinal mucosa of rats at pH 2 and 6.8 in the donor chamber at 37°C.

Test compound	Tissue	pH value	Apparent permeability coefficient [P_{app} (cm/sec), mean ± SD; $n=3$]
Sodium fluorescein 0.001%/2% DMSO	Glandular stomach	pH 2	$3.3 \pm 1.5 \times 10^{-7}$
		pH 6.8	$2.4 \pm 0.6 \times 10^{-6}$
	Small intestine	pH 6.8	$9.5 \pm 1.1 \times 10^{-6}$
Bacitracin-FITC 0.1%	Glandular stomach	pH 2	$1.4 \pm 0.6 \times 10^{-7}$
		pH 6.8	$7.6 \pm 0.7 \times 10^{-7}$
	Small intestine	pH 6.8	$3.0 \pm 0.5 \times 10^{-6}$
Propranolol HCl 200 µmol/L/2% DMSO	Glandular stomach	pH 2	$5.9 \pm 0.3 \times 10^{-7}$
		pH 6.8	$1.1 \pm 0.7 \times 10^{-6}$
	Small intestine	pH 6.8	$5.2 \pm 1.6 \times 10^{-6}$
Cimetidine 2 mM/2% DMSO	Glandular stomach	pH 2	$1.4 \pm 0.4 \times 10^{-5}$
		pH 6.8	$9.6 \pm 2.3 \times 10^{-6}$
	Small intestine	pH 6.8	$2.8 \pm 0.5 \times 10^{-5}$

Indicated values are means of three experiments (mean ± SD; $n=3$).

cimetidine, a low-permeability drug. We set out to collect information about the leakiness of the paracellular space. Marked differences in the P_{app} values for propranolol HCl and cimetidine were observed. Figures 7 and 8 highlight the absorption rate of the hydrophilic beta blocker and of the H_2 antagonist through gastric and intestinal mucosa, respectively. The permeability of propranolol HCl through gastric mucosa was determined to be $5.9 \pm 0.3 \times 10^{-7}$ at pH 2 and $1.1 \pm 0.7 \times 10^{-6}$ at pH 6.8, respectively. In contrast, the P_{app} value of cimetidine through gastric mucosa stands at $1.4 \pm 0.4 \times 10^{-5}$ at pH 2 and $9.6 \pm 2.3 \times 10^{-6}$ at pH 6.8. The findings of this study support the assumption that the paracellular space lost its integrity owing to acidic conditions on the AP side. However, we have determined a 5-fold higher absorption rate for cimetidine compared with propranolol HCl through intestinal mucosa at pH 6.8. This was observed despite the fact that cimetidine is a low permeability and low solubility drug. One possibility for the low transport of propranolol HCl is mentioned in the work of Pretorius and Bouic (2009). The authors claimed factors influencing permeability of propranolol HCl *in vitro* compared with *in vivo*, since propranolol HCl is transported transcellularly. Owing to a flow-through diffusion method, the mean flux values of propranolol HCl were considerably higher than the permeability of drug classes 3 according to the biopharmaceutics classification system (BCS). Their results are in contrast with other authors who found that propranolol exhibits a low *in vitro* permeability. Further considerations are decreasing the pH of the solution of a weak base can decrease the ratio of unionized to ionized species, which in turn can lead to poor transmucosal permeation. Wang et al. (2010) reported that optimal mucosal permeation of propranolol HCl could be achieved at pH of 7.4. Additionally, our data of cimetidine is concordant with those reported by Mummaneni et al. (1995). The authors found high gastric pH resulted in higher plasma concentrations of cimetidine in beagle dogs ($C_{max} = 8.22 \pm 0.97$ at pH ≤ 3, $C_{max} = 14.51 \pm 0.96$ at pH ≥ 5 (µg/mL)). They concluded

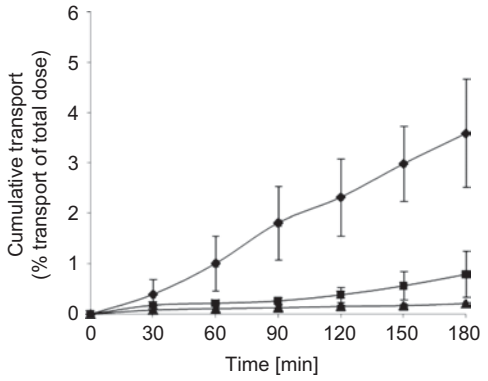

Figure 7. Transport of propranolol HCl 200 µmol/L; small intestinal mucosa pH 6.8 (♦), glandular stomach pH 6.8 (■), glandular stomach pH 2 (▲). All experiments were performed in an atmosphere of 95% O_2 and 5% CO_2 at 37°C. Indicated values are means of three experiments (mean ± SD, $n=3$).

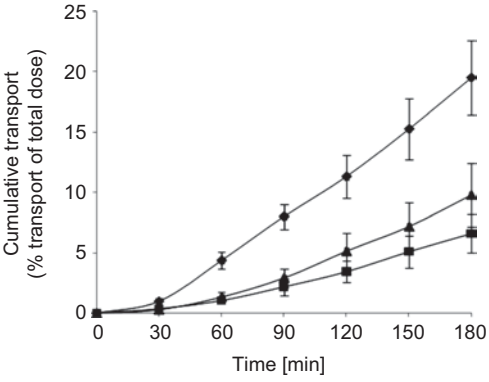

Figure 8. Transport of cimetidine 2 mM; small intestinal mucosa pH 6.8 (♦), glandular stomach pH 6.8 (■), glandular stomach pH 2 (▲). All experiments were performed in an atmosphere of 95% O_2 and 5% CO_2 at 37°C. Indicated values are means of three experiments (mean ± SD, $n=3$).

that changes in gastric pH also resulted in variations in the apparent kinetics of absorption.

To take in consideration the pH-dependent lipophilicity of model compounds, the log*D* values were

determined at pH 2 and 6.8 of NaFlu and bac-FITC. Results are listed in Table 3. According to these results, both model compounds showed much higher lipophilicity at pH 2, which should strongly improve their membrane permeability. On the contrary, results of permeation studies showed opposite permeation behavior. The P_{app} of both NaFlu and bac-FITC was >5-fold higher at pH 6.8 than at pH 2 indicating that other factors seem to have a much greater impact on pH-dependent gastric drug uptake than their lipophilic character. Whereas the pH in the donor chamber increased from 2 up to 6.1 within 3 h, the P_{app} values of bacitracin and fluorescein did not increase accordingly. Propranolol HCl has an octanol/water partition coefficient of –0.45 and cimetidine of 0.40 (Table 3). Both model compounds are weak bases. As a consequence at higher pH, a higher fraction of nonionized drug, a more readily absorbed form, could be present. Palm and coworkers showed a pH dependency on permeability of alfentanil and cimetidine across Caco-2 cell monolayers. For both drugs, a linear increase in permeability was observed with increasing unionized fraction (Palm et al., 1999). Our findings differ from these data, P_{app} value of cimetidine was determined to be 1.5-fold higher at pH 2 than at pH 6.8 through gastric mucosa. This observation underlines the presumption of the leakiness of the paracellular space at pH 2 on the AP side.

By comparing the uptake of all compounds from gastric and intestinal mucosa, as shown in Table 2, a significantly higher absorption rate from the intestinal mucosa becomes obvious. These results are in good agreement with previous studies. Kimura and Higaki (2002) reported that propanolol and *N*-methyltyramine show an absorption rate constant of 1.45 (h^{-1}) and 0.11 (h^{-1}) in the stomach and 5.88 (h^{-1}) and 1.47 (h^{-1}) in the duodenum, respectively. Impedance analysis on mouse gastric and small intestinal mucosa revealed an epithelial resistance of 33 ± 6 Ω cm^2 and 23 ± 5 Ω cm^2, respectively (Schulzke et al., 2005). Another pH-dependent drug absorption was reported by Moazed and Hiebert (2009). The authors found out that acidic environment favors the transport of unfractionated heparin, a very large and highly acidic molecule, through gastric mucosa, while low-molecular-weight heparin shows higher movement across intestinal mucosa. Within this study, however, we could demonstrate that independently from the log*D* the pH has a great impact on gastric drug uptake, as well as on the transport route of the drug.

Table 3. Experimentally determined log*D* values from sodium fluorescein and bacitracin-FITC at pH 2 and 6.8.

	pH 2	pH 6.8
Sodium flluorescein	1.47 ± 0.03	−0.35 ± 0.02
Bacitracin-FITC	0.50 ± 0.33	−1.46 ± 0.06
Propranolol HCl*		−0.45
Propranolol*		3.56
Cimetidine*		0.40

Indicated values are means of three experiments (mean ± SD; $n = 3$).
*Log*P* values obtained from literature (Craig, 1990).

Conclusion

Within this study, permeation experiments with freshly excised gastric mucosa were performed in Ussing chambers applying to the solution of various pH gradients. Histological investigations demonstrated that the tissue remains viable to acidic exposure. In addition, the gastric tissue is capable of maintaining a pH gradient of 1–1.2 in the donor chamber and pH 7–8 in the acceptor chamber over this time period. A significant impact on drug uptake was shown due to the pH in the donor compartment. Furthermore, it is highly recommended to perform *in vitro* gastric permeation studies also at low pH values in the donor compartment.

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Declaration of interest

The authors report no declarations of interest.

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