INFLUENCE OF SHORT CHAIN FATTY ACIDS ON THE ABSORPTIVE CLEARANCE OF RANITIDINE HCl FROM RABBIT INTESTINE

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يعتبر هيدروكلوريد الرانتيدين من مضادات مستقبلات الهستامين الموجودة فى المعدة والذى يؤدى استخدامه الى خفض افراز الحمض فى المعدة فى أثناء النهار أو أثناء النوم. ويمتص هذا المركب بنسبة   % بعد أخذه عن طريق الفم. وقد تم اجراء هذا البحث لاختبار تأثير الأحماض الدهنية قصيرة السلسلة وهى الأحماض ثنائية وثلاثية ورباعية الكربون على معامل امتصاص مركب هيدروكلوريد الرانتيدين من كل من الأمعاء الدقيقة والقولون الصاعد فى الأرنب وذلك باستخدام أسلوب التروية فى أمعاء الأرنب. تم ضخ كل من هذه المركبات بمفرده بتركيز   عيارى مركب هيدروكلوريد الرانتيدين بتركيز   ,   عيارى وقد أظهرت النتائج أن معامل امتصاص مركب هيدروكلوريد الرانتيدين فى القولون الصاعد يساوى تقريبا ضعف معامل امتصاصه فى الأمعاء الدقيقة. وكانت هناك علاقة قوية بين معامل امتصاص مركب هيدروكلوريد الرانتيدين واند الماء عبر جدران الأمعاء فى كل من الأمعاء الدقيقة والقولون الصاعد مما يوضح أن آلية امتصاص هذا المركب هى الانتشار السلبى عبر الفراغات البين خلوية فى جدار الأمعاء. ولقد انعكس الامتصاص الغير كامل لهذا المركب من القيمة السلبية للطول التشريحى اللازم لامت كل من الأمعاء الدقيقة والغليظة على السواء. ولقد أثبتت التجارب أن الأحماض الدهنية قصيرة السلسلة لها تأثير ايجابى على امتصاص مركب هيدروكلوريد الرانتيدين وكان هذا التأثير كما يلى: الأحماض رباعية الكربون أكبر من الأحماض ثلاثية الكربون أكبر من الأحماض ثنائية الكربون. وقد كان التأثير الايجابى للأحماض الدهنية قصيرة السلسلة على معامل امتصاص مركب هيدروكلوريد الرانتيدين فى القولون الصاعد أكبر من الأمعاء الدقيقةوذلك لأن انسياب الماء وكل من أيونات الصوديوم والكلور أعلى فى القولون الصاعد منه فى الأمعاء الدقيقة. من هذا البحث يمكن استنتاج أن هناك اختلافات موضعية فى امتصاص مركب هيدروكلوريد الرانتيدين من كل من الأمعاء الدقيقة والغليظة والذى يمكن زيادة امتصاصه ايجابيا عن طريق اضافة الأحماض الدهنية قصيرة السلسلة.

Ranitidine HCl is a histamine H_2-receptor antagonist reducing gastric acid secretion under daytime and nocturnal basal conditions. Ranitidine HCl is 50% absorbed after oral administration. This research was undertaken in order to examine the effect of short-chain fatty acids (SCFAs), acetate, propionate, and butyrate on the absorptive clearance of ranitidine HCl as a function of intestinal site (jejunoileum vs ascending-colon). A "through-and-through" in situ intestinal perfusion technique was adopted using the rabbit as an animal model. Coperfusion of either sodium acetate, sodium propionate, or sodium butyrate, 25 mM each, along with ranitidine HCl, 0.2 mM, allowed for an examination of increased solvent drag on intestinal permeability of this compound in both anatomical sites. The results show that ranitidine HCl is absorbed from rabbit jejunoileum as well as the ascending-colon, however the value of the absorptive clearance of this compound normalized to the intestinal length PeA/L in the ascending-colon was almost double that in the jejunoileum. A strong correlation was found between the absorptive clearance and the net water flux in both segments suggesting that the mechanism of ranitidine HCl absorption apparently consists of passive diffusion via the paracellular pathway. The negative value of anatomical reserve length ARL in both segments reflects the incomplete absorption of this compound. SCFAs had a significant effect on increasing the absorptive clearance of ranitidine HCl in both segments studied. This effect was in the order butyrate > propionate > acetate. However there was no statistical difference between the effect of butyrate and propionate. The permeability enhancing effect of SCFAs was much higher in the ascending-colon, this could be attributed to the higher Na^+, Cl^-, and water

influx in this segment. In conclusion, marked segmental differences in the absorption of ranitidine HCl are apparent in the rabbit small and large intestine which could be significantly enhanced by the use of SCFAs.

INTRODUCTION

Short chain fatty acids, SCFAs are produced during fermentation of dietary fibers by colonic bacteria. Absorption of the SCFAs, acetate, propionate, and butyrate occurs primarily by nonionic diffusion and paracellular absorption in the proximal colon.[1] However, an additional $SCFA^-/HCO_3^-$ exchange mechanism is present in the luminal membrane of rat colonic epithelial cells.[2-4] Absorption of SCFAs not only serves as an additional energy supply for colonic epithelial cells, but it has also a significant impact on NaCl and water absorption.[5-7] SCFAs stimulate electroneutral uptake of Na^+, presumably by acidification of colonocytes and activation of apical Na^+/H^+ exchangers.[8] Cl^- absorption is stimulated by increased HCO_3^- production during SCFAs metabolism. Another model is reported in which butyrate is taken up via nonionic diffusion or $SCFA^-/HCO_3^-$ exchange. Subsequently electroneutral NaCl absorption is activated by parallel Cl^-/butyrate and Na^+/H^+ exchange.[9] Thus SCFAs transport is an important factor that regulates colonic fluid balance and absorption of NaCl and water from the intestine. The increased net water flux associated with absorption of SCFAs would enhance the absorption of polar compounds due to solvent drag effect via the paracellular pathway.The major objective of the present study was to examine the influence of increased chain length of short chain fatty acids (SCFAs) on the effective permeability surface area product normalized to the intestinal length (PeA/L), of the H_2-receptor antagonist, ranitidine HCl, using single pass intestinal perfusion technique, *in situ*, in the rabbit.[10-12] Another important secondary objective of this study was to estimate the membrane transport parameters of ranitidine HCl in both the jejunoileum and ascending colon of the rabbit intestine and integrate such estimations with the net water flux to establish a baseline correlation between the solvent drag effect and

intestinal absorption of this drug. Ranitidine HCl was selected as a model polar drug having relatively, low oral bioavailability. Ranitidine hydrochloride is a histamine H_2-receptor antagonist. it occurs as a white to pale yellow granular substance having a slightly bitter taste and sulfur-like odor, the drug has a pK_a of 2.7, and a pk_b of 8.2, and solubilities of 660 mg/mL in water and 190 mg/mL in alcohol respectively,[13,14] Ranitidine competitively inhibits the action of histamine on the H_2 receptors of parietal cells, reducing gastric acid secretion under daytime and nocturnal basal conditions and also when stimulated by food, histamine, pentagastrin,[13] insulin, and amino acids.[15] Ranitidine HCl is 50% absorbed after oral administration, compared to an iv-injection with mean peak levels of 440 to 545 ng/ml occurring at 2 to 3 hours after a 150-mg dose. The plasma profile of healthy volunteers after oral or intravenous administration of the drug presents at least two peaks.[16-18] Several authors considered the existence of secondary peaks as a result of discontinuous absorption from specific sites along the gastrointestinal tract.[19-21]

MATERIALS AND METHODS

A- Materials

Ranitidine HCl, caffeine, sodium acetate, sodium propionate, and sodium butyrate, were all purchased from Sigma chemical Co. (St.Louis, MO, USA). Monobasic ammonium phosphate and phosphoric acid were obtained from Merck, Darmstadt, Germany. Acetonitrile HPLC-grade was obtained from BDH Laboratory, England. Sodium chloride 0.9% for injection, USP was obtained from El-Nasr Pharmaceutical Chemicals Company, Egypt. Ketamine HCl (100 mg/ml) was obtained From EIPICO Pharmaceutical Company, Egypt. Chlorpromazine HCl (25 mg/ml) was obtained From Misr Pharmaceutical Company, Egypt. All chemicals were of analytical reagent grade.

B- Equipment

Peristaltic pump (LKB-Produkter AB S-16125 Bromma, Sweden), Timer, pH meter (Digi-Sense®, Cole-Parmer Instrument Company, Niles, IL 60714, USA), Water bath (Gerhardt®, H. Jurgens & Co.-GmbH & Co.-Bremen, Germany), Centrifuge (Minor 35, M.S.D., L.T.D., England). High pressure liquid chromatograph (Waters™ 600 controller, USA) equipped with a variable wavelength detector (Waters™ 486, Tunable Absorbance Detector, USA) and an automatic sampling system (Waters™ 717 Plus Autosampler, USA) and the whole equipment is under computer control. Reversed phase column 15 cm X 3.9 mm (i.d.) C_{18}, μ Bondapak™, Waters, with an average particle size of 10 μm.

C- Preparation of the drug solutions for intestinal perfusion

The perfusion solutions containing 0.2 mM ranitidine HCl were prepared by simply weighing the exact amount of the drug and dissolving it in 0.9% sodium chloride for injection, USP. In the experiments that involved coperfusion of SCFA as an absorption enhancer, ranitidine HCl was dissolved in 0.9% sodium chloride for injection, USP containing 25 mM of either sodium acetate, sodium propionate or sodium butyrate. All the solutions were freshly prepared on the day of the experiment and sonicated for 30 minutes to ensure complete dissolution of the drug and enhancer.

D- Segment preparation

Male albino rabbits weighing 2.8-3.1 kg were utilized in these experiments. Prior to surgery, the rabbit was fasted over night. The animal was then anesthetized by intramuscular injections of ketamine HCl, given in two doses each of 45 mg/kg at 15 minutes interval and a third dose of 25 mg/kgm, 15 minutes later. Chlorpromazine HCl was used as muscle relaxant (two doses of 2 mg/kgm given i.m at 15 min interval). The injections were given rapidly and deeply into the thigh muscle.

After induction of anesthesia the rabbit was laid in a supine position on an underpad, which was placed over a heating pad to maintain body temperature throughout the experiment. The abdominal area was shaved and cleaned with a depilatory cream, a longitudinal incision 6-8 cm was made. The intestinal segments of interest were exposed and isolated carefully. In order to cannulate the jejunoileum segment, the proximal end was tied off using surgical silk, then it was cannulated using a 3-way stopcock cannula. The desired length of the intestinal segment was then measured by a premeasured thread, and the distal end was cannulated using an L-shaped glass cannula.

For the colon, the proximal end was tied off immediately after the ampulla coli, the desired length (15 cm) was measured adopting the same procedures, and finally the distal end tied off. Two incisions were made, one on each end, and the solid fecal debris was squeezed out by gentle manipulation of the segment. The rest of the fecal debris was forced out by gently infusing 37° normal saline through the proximal end. Finally, both the proximal and distal ends were cannulated as described before. The isolated segment was kept warm and moist by frequent application of 37° normal saline to a gauze pad covering the intestine. The remainder of the intestine was returned into the abdominal cavity for better maintenance of the temperature. The isolated segments were kept in a horizontal level throughout the experiment to avoid the hydrostatic pressure which could affect the fluid movement across the intestinal membrane. At the end of the experiment the animal was euthanized by injecting an overdose of sodium pentobarbital through the marginal ear vein. The intestinal segments under study were excised and an exact measure of the length of these segments was done by placing each segment on a ruler wetted with normal saline. This length was used for calculation of the membrane transport parameters of ranitidine HCl.

E- Experimental design

Four groups, each consisted of three male, albino rabbits weighing 2.8-3.1 kg were used in these experiments. The first group was utilized to estimate the membrane transport parameters of ranitidine HCl in both the jejunoilum and ascending-colon of the rabbit intestine. The intestinal perfusion was conducted in both segments in the same time in order to allow for comparison between parameters under identical conditions. The other three groups were

utilized to examine the effect of SCFAs (sodium acetate, sodium propionate, and sodium butyrate) on the absorptive clearance of ranitidine HCl from both segments. The drug concentration was 0.2 mM, while the concentration of each enhancer was 25 mM, which was coperfused alone with the drug (n=3).

F- *In Situ* intestinal perfusion

Solutions containing ranitidine HCl in normal saline, as described before were perfused at a flow rate of 0.37 ml/min. The intestinal effluent samples were collected at 10-minutes intervals for 120 minutes in 10-ml preweighed stoppered tubes. These tubes were weighed again after sample collection, and the effluent weight was recorded as the difference. Intestinal net water flux was estimated gravimetrically and effluent concentrations were corrected accordingly.

G- Chromatography
Standard solutions

A 7-point standard curve was prepared by adding appropriate volumes of ranitidine HCl stock solution in methanol into a series of 13-ml ground glass stoppered centrifuge tubes in the amount of 0.1, 0.5, 1, 5, 10, 20, and 50 µg. Each of these tubes was spiked with 7.0 µg of caffeine as an internal standard. The methanol was evaporated off and the residue was reconstituted in one ml of mobile phase, vortex-mixed, and 200 µl were transferred to microvials which were loaded on automatic sampler after crimping for injection.

Samples

The perfusate samples collected during the intestinal perfusion were centrifuged for 5 minutes in order to precipitate any mucus debris. 200 µl of the supernatant as well as the solutions remaining after intestinal perfusion were withdrawn and added to test tubes spiked with 7.0 µg internal standard, caffeine. Another 800 µl of the mobile phase was added and the tubes were vortexed for 1 minute. 200 µl were then transferred to microvials which were loaded on the automatic sampler after crimping to avoid evaporation of the solvent. 40 µL of these samples together with standards were injected. Isocratic separation was achieved using a 15 cm X 3.9 mm (i.d.) C_{18}, µ

Bondapak^{TM}, Waters, reversed phase column with an average particle size of 10 µm and a mobile phase consisted of 10 mM monobasic ammonium phosphate dissolved in water-acetonitrile (90:10). The pH was adjusted to 6.0 by addition of phosphoric acid. The flow rate was 1.2 ml/min and the temperature was ambient. The effluent samples were detected at 228 nm. Under these conditions the retention time of the drug and internal standard was 2.51 and 4.95 minutes, respectively. Figure 1 shows a typical chromatogram obtained during the analysis of perfusate samples containing ranitidine HCl. Analysis of the solutions remaining after intestinal perfusion was done in order to determine the inflow concentration accurately. The peak areas for ranitidine HCl and caffeine were calculated with the millennium[32] software. The calibration curves were constructed from the drug concentrations and peak area ratios of the drug/internal standard. The peak area ratios for the unknown samples were used to calculate the unknown concentrations from the standard calibration curves.
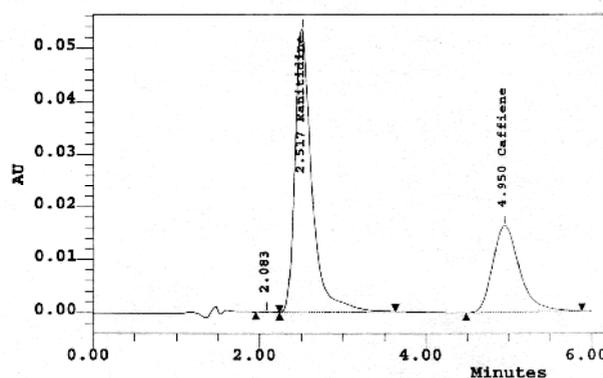
Fig. 1: Typical chromatogram obtained during the analysis of perfusate samples containing ranitidine HCl. The retention time for ranitidine and caffeine (IS) was 2.51, and 4.95 minutes, respectively.

H- Data analysis
Absorptive clearance

The flow rate was estimated for each perfusion experiment from the linear regression of the volume remaining during perfusion versus time. The volume of the outflow samples was estimated gravimetrically taking the density of the aqueous samples as 1.0, the same as water. From the difference in flow rate

entering and leaving the intestinal segment, the outflow concentration was corrected for net water flux. The ratio between the corrected concentration at the outflow (C_1) and that at the inflow (C_0) was calculated for each perfusate sample collected. The average of the outflow-to-inflow concentration ratios for the fractions collected from 70 to 120 min was taken as the steady-state ratio. This ratio at steady-state is given by:[22-24]

$$\left\{\frac{C_1}{C_0}\right\}_{ss} = \exp^{-\left(\frac{Pe.A}{Q}\right)} \tag{1}$$

where C_1 is the corrected concentration leaving the intestinal segment, C_O is the concentration entering the segment, $\{C_1/C_0\}_{ss}$ is the fraction remaining to be absorbed at steady-state, A is the effective surface area (cm^2), Pe is the apparent permeability coefficient (cm/min), and Q is the bulk perfusate flow rate (mL/min) within the intestinal segment.

Rearrangement of Equation (1) allows the permeability-area product (Pe*A) to be calculated. The (Pe*A) could be considered to be the absorptive clearance (mL/min):

$$PeA = -Q \ln\left(\frac{C_1}{C_0}\right)_{ss} \tag{2}$$

After correction for the net water flux:

$$PeA = -Q*\ln\left\{\frac{\left(C_{(out)}*Q_{(out)}\right)}{\left(C_{(in)}*Q_{(in)}\right)}\right\}_{ss} \tag{3}$$

where $Q_{(in)}$ is the flow rate entering the intestinal segment and $Q_{(out)}$ is the flow rate leaving it. Since $\{(C_{(out)}*Q_{(out)})/(C_{(in)}*Q_{(in)})\}_{ss}$ is the fraction remaining after solution has passed through the intestinal length (l), then the fraction absorbed is:

$$Fa = 1 - \left\{\frac{\left(C_{(out)}*Q_{(out)}\right)}{\left(C_{(in)}*Q_{(in)}\right)}\right\}_{ss} = 1 - \exp^{-(PeA/Q)} \tag{4}$$

The anatomical reserve length (ARL) was given by:[24]

$$ARL = (L^*) - (l^*) \tag{5}$$

where, ARL is defined as the length, (cm) of the intestine remaining after absorption has been completed for a given solute. L* is the maximal intestinal length available for absorption. l* is the intestinal length along which absorption is complete, (cm). An arbitrary small fraction of solute remaining in the lumen was considered as the criteria for complete absorption.[24] Taking this fraction as 5%, and replacing in equation (1) will give the following equation:

$$0.05 = \exp^{-\{(PeA.l^*)/Q\}} \tag{6}$$

where, PeA is the effective permeability surface area product normalized to length. l* is the length required for 95% absorption (L95% ab.) of a given solute.

Effect of solvent drag on intestinal absorptive clearance

The influence of water flux on the absorption of the drugs across the intestinal membrane was studied by plotting the absorptive clearance versus the net water flux J_w, where J_w (ml/min) is given by:

$$J_w = Q_{in} - Q_{out} \tag{7}$$

The net amount of drug absorbed per unit time can be described as the sum of two terms, the diffusive contribution and the convective contribution corresponding to the solvent drag effect. The net amount of drug absorbed per unit time is then given by:[25-27]

$$J_s = K_s\left(C - C_p\right) + \Phi_s J_w C \tag{8}$$

In which, the first term on the right is diffusive and the second is convective and, J_s, is the rate of absorption of the solute from the lumen (µg/min). Ks is the diffusive permeability coefficient given by $DAK_p/\Delta x$. In which D, is the diffusion coefficient of the solute, A, is the effective surface area, K_p, is the partition coefficient of the solute, and Δx, is the path length. C, C_p are the solute concentrations in the lumen and plasma, respectively. Φ_s, is the sieving coefficient of the given compound, represents the ratio between the concentration of the compound in the convective stream to that in the luminal fluid. J_w, is the rate of water flux within the intestinal segment. At the steady state, due to sink conditions in the blood, equation (8) is reduced to:

$$J_{ss} = \frac{DAK_p}{\Delta X}\left(C_{ss}\right) + \Phi J_w\left(C_{ss}\right) \qquad (9)$$

where, J_{ss} is the steady state solute flux (μg/min) and C_{ss} is the length averaged steady state concentration of the solute in the lumen (μg/ml). Rearrangement of equation (9) gives:

$$\frac{J_{ss}}{C_{ss}} = \frac{DAK_p}{\Delta X} + \Phi J_w \qquad (10)$$

The term J_{ss}/C_{ss} represents the overall absorptive clearance of the given solute (ml/min), regardless its route or mechanism.

Statistical analysis

The membrane transport parameters of ranitidine HCl were determined in both the jejunoileum and ascending-colon of the rabbit intestine when ranitidine HCl was perfused alone or in presence of the SCFAs as absorption enhancers. The Student's t-test was utilized to compare between pharmacokinetic parameters obtained in both segments when ranitidine HCl was perfused alone or in combination with the SCFAs in order to determine if coadministration of the SCFAs improve the intestinal absorption of ranitidine HCl in the rabbit. P-value < 0.05 was considered significant.

RESULTS AND DISCUSSION

Segmental differences in the absorption of ranitidine HCl from rabbit intestine

Ranitidine HCl was incompletey absorbed from the rabbit small and large intestine. The membrane transport parameters of this compound were estimated and summarized in Table 1. The length required for complete absorption of this compound (L 95%) reaches (396±51 cm) in the jejunoileum and (234±6 cm) in the ascending colon, this means that complete absorption of this compound occurs hypothetically beyond the whole length of the intestine in both regions and the absorption of this compound is incomplete from the small and large intestine. These results are consistent with previous oral bioavailability studies of ranitidine HCl in humans which indicated that the oral bioavailability of this compound is incomplete.[18,28] The absorptive clearance

normalized for the intestinal length (PeA/L, ml/min cm) indicated that the absorption of ranitidine HCl is higher in the ascending colon compared with the jejunoileum. The PeA/L value in the ascending colon was (0.0058 ± 0.0002), which is almost double that in the jejunoileum (0.0028±0.0004) ml/min cm (P-value <0.05). This was reflected as double amount absorbed per unit length in the ascending-colon compared with the jejunoileum, where the percentage fraction absorbed per unit length (%Fa/cm) was (1.63±0.07) in the ascending-colon compared with (0.75±0.07) in the jejunoileum. The delayed absorption of ranitidine HCl from the ascending colon may account for the presence of a secondary peak in the plasma profile of healthy volunteers after oral administration of the drug.[16-18] Several authors considered the existence of secondary peaks as a result of discontinuous absorption from specific sites along the gastrointestinal tract.[19-21] Site-dependent small intestinal absorption of ranitidine in healthy, male volunteers was repoted.[19] The absorption rates calculated from intestinal samples showed distinct site-dependence, the highest rates were found in the most proximal region (duodenojejunal junction), and the most distal perfusion sites (distal ileum). In both of these regions there was a significant positive correlation between the net intestinal water flux and the movement of ranitidine. Within the mid-jejunum, every subject showed marked secretion of ranitidine into the gut lumen, and in this region there was no influence of water flux on ranitidine movement. The intraluminal results were confirmed by the corresponding site-dependent areas under the serum concentration-time curves (AUC), which decreased with the distance of the perfusion site from the teeth. The results demonstrated that the small intestine is a site of a gradient of absorption of ranitidine. In another study that was conducted on rats,[29] the results showed that ranitidine absorption from the lower ileum contributes significantly to systemic ranitidine concentrations before and during the time of the first concentration maximum. Separation of the drug mass into multiple boluses may contribute to secondary peaks in ranitidine concentration-time profiles.

Table 1: Membrane transport parameters of ranitidine Hcl from the rabbit small and large intestine.

Rabbit Iden.	PeA/L ml/min.cm	R(out)/R(in)	%Fa	%Fa/cm	PeA ml/min	L (95%ab) cm
Jejunoileum						
R1	0.0035	0.6662	33.38	0.88	0.1076	300.16
R7	0.0023	0.8071	19.29	0.62	0.0707	474.82
R8	0.0026	0.7844	21.56	0.74	0.0796	415.61
mean	0.0028	0.7526	24.74	0.75	0.0860	396.86
±SE	0.0004	0.0437	4.37	0.07	0.0111	51.28
Ascending colon						
R1	0.0062	0.8689	13.11	1.64	0.0496	221.76
R7	0.0054	0.8355	16.45	1.50	0.0595	238.81
R8	0.0058	0.8259	17.41	1.74	0.0642	241.70
mean	0.0058*	0.8434	15.66	1.63	0.0578	234.09
±SE	0.0002	0.0130	1.30	0.07	0.0043	6.22

Statistically different from jejunoileum, P-value < 0.05.

Contribution of the solvent drag to the absorptive clearance of ranitidine HCl from rabbit small and large intestine

Ranitidine HCl is relatively polar compound with a solubility of 660 mg/ml in water.[13] The solvent drag appears to influence its absorptive clearance to a major extent through the paracellular route. Figure 2, indicates that the absorptive clearance (PeA/L, ml/min cm)) of this compound increased linearly as a result of increasing net water flux (Jw/L, ml/min cm), this effect was more pronounced in the ascending colon compared with the jejunoileum due to the higher water flux in this segment. According to Lifson's model [25, 26] equation (10) represents the transcellular and paracellular contributions to the overall absorptive clearance of a given compound. Fitting the linear regression equations obtained in Figure 2 to this model demonstrated clearly that the drug is dependent entirely on the convective component through the paracellular route in its absorption. The transcellular diffusive component of this compound is zero as indicated from the negative intercept in the jejunoileum and ascending colon (the intercept is statistically not different from zero). The importance of solvent drag on the absorption of numerous drugs through the paracellular pathway had been well established.[30-33] The relative contributions of the diffusive (DKA/ x) and convective (Jw) clearances to the overall absorptive clearance (Eq. 10) were estimated from the linear regression equation obtained in Figure 2. The average of the overall absorptive clearance (DKA/ x + Jw) in the jejunoileum is (0.0028 ± 0.0004), while that in the ascending colon is (0.0058 ± 0.0002) ml/min cm, the intercept obtained from the linear regression equation (Figure 2) is considered to be zero (Statistically not different from zero), which represents the transport via the transcellular pathway (DKA/ x). This means that 100% of the absorptive clearance is represented by the paracellular convective component (Jw).
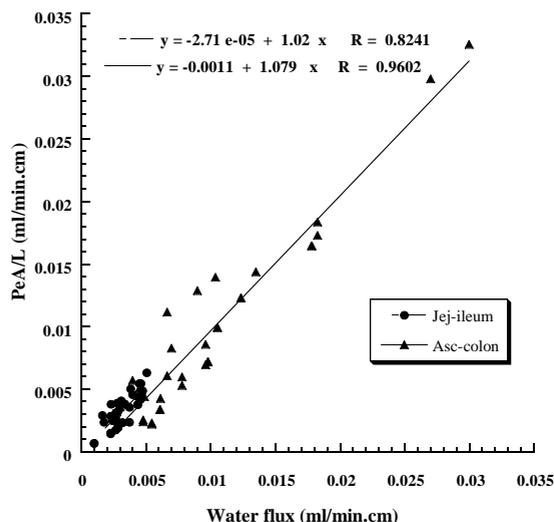
Fig. 2: Effect of net water flux on the absorptive clearance of ranitidine HCl from the jejunoileum and ascending-colon of rabbit intenstine (n= 3).

The sieving coefficient of ranitidine HCl ( ) obtained from the linear regression equation (Figure 2), is almost 1.0 in both the jejunoileum and ascending colon indicating a high extent of interaction with water for this compound. Since one of the major physiological functions of the ascending colon is the absorption of water and sodium,[34] thus the entrainment of ranitidine HCl in the stream of influxed water represents the major transport mechanism in this segment through the paracellular pathway, this was reflected as higher correlation value in the ascending colon compared with the jejunoileum (R = 0.96 in ascending colon versus 0.82 in the jejunoileum, Figure 2). On the other hand the anatomic radius of the colonic segment is higher compared with the jejunoileum, applying the same volumetric flow rate (0.37 ml/min) in both segments during perfusion results in a slower linear flow velocity (β) in the ascending colon compared with the jejunoileum. Since the linear flow velocity (β) is correlated with the volumetric flow velocity (Q) by the formula:[35]

$$\beta = Q / \quad r^2 \qquad cm/min \qquad (11)$$

Thus the mean residence time in the ascending colon is higher compared with the jejunoileum, which allows for higher value of the absorptive clearance, (PeA/L) in this

segment which is almost double that in the jejunoileum, (0.0058 ± 0.0002) versus (0.0028 ± 0.0004) ml/min cm. (P-value < 0.05). In a study conducted on Caco-2 cell monolayers,[36] the apparent permeability coefficient (P_{app}) value for ranitidine was increased by 15- to 20-fold in a calcium-free medium or in the transport medium containing EDTA, indicating that the paracellular passive diffusion is the rate determining pathway for the transport of ranitidine. The apparent permeability coefficients (P_{app}) were unchanged throughout the concentration range studied, indicating a passive diffusion pathway across intestinal mucosa. In another study conducted on rats[37] the uptake rate of ranitidine from the jejunum and colon was linear with a concentration range of 0.0005-5 mM, and there was no competition for uptake by either famotidine or cimetidine. The results indicated that uptake of ranitidine in the rat jejunum and colon occurs by a predominantly passive process and ranitidine exhibited regional differences in uptake rate in both segments.

Effect of short chain fatty acids (SCFAs) on membrane transport parameters of ranitidine HCl from rabbit intestine

The effect of coperfusion of SCFAs on the membrane transport parameters of ranitidine HCl from the jejunoileum and ascending colon of the rabbit intestine is depicted in Tables 2 and 3, respectively. All the three compounds had increased the absorptive clearance (PeA/L) of ranitidine HCl from both segments, however the effect of both sodium butyrate and sodium propionate was statistically different from the control (P-value < 0.05), while that of sodium acetate was statistically insignificant (P-value >0.05). Sodium butyrate exerted the highest enhancing effect on both the absorptive clearance normalized to the intestinal length (PeA/L) and the percentage fraction absorbed per unit length (%Fa/cm) of ranitidine HCl. The values of these parameters were almost double compared with the control in both the anatomical sites studied (Tables 2 and 3). Sodium propionate had a significant enhancing effect on both parameters in both segments studied compared with the control (P-value < 0.05), however there was no statistical difference between the effect of butyrate and propionate. Sodium acetate had also

Table 2: The Effect of SCFAs Coperfusion on Membrane Transport Parameters of Ranitidine HCl in the jejunoileum of the Rabbit intestine.

Parameter	Control Mean (±SD, n=3)	+Acetate* Mean (±SD, n=3)	+Propionate* Mean (±SD, n=3)	+Butyrate* Mean (±SD, n=3)
Absorptive clearance, PeA/L (ml/min.cm)	0.0028 (0.0004)	0.0038** (0.0004)	0.0042*** (0.0002)	0.0060*** (0.0011)
Fraction remaining to be absorbed R(out)/R(in)	0.7526 (0.0437)	0.7425 (0.0387)	0.6353 (0.0131)	0.4955 (0.0405)
% Fraction absorbed (%Fa)	24.74 (4.37)	25.78 (3.87)	36.47 (1.31)	50.45 (4.05)
% Fraction absorbed normalized to length (%Fa/cm)	0.75 (0.07)	0.9567 (0.0915)	1.06 (0.0089)	1.39 (0.2246)
Length required for complete absorption (L95%), (cm)	396.86 (51.28)	295.31 (29.14)	234.65 (12.46)	176.73 (39.83)
Anatomical reserve length (ARL), (cm)	-246.86 (51.28)	-145.31 (29.14)	-84.66 (12.46)	-26.73 (39.83)

 * Sodium salt at 25 mM concentration.
 ** Statistically not different from control P-value > 0.05.
 *** Statistically different from control P-value < 0.05.

Table 3: The effect of SCFAs coperfusion on membrane transport parameters of ranitidine HCl in the ascending-colon of the rabbit intestine.

Parameter	Control Mean (±SD, n=3)	+ Acetate* Mean (±SD, n=3)	+Propionate* Mean (±SD, n=3)	+Butyrate* Mean (±SD, n=3)
Absorptive clearance, PeA/L (ml/min.cm)	0.0058 (0.0002)	0.0083** (0.0009)	0.0111*** (0.0017)	0.0128*** (0.0003)
Fraction remaining to be absorbed, R(out)/R(in)	0.8434 (0.0130)	0.7221 (0.0147)	0.6755 (0.0074)	0.5945 (0.0282)
% Fraction absorbed (%Fa)	15.66 (1.30)	27.79 (1.47)	32.45 (0.7370)	40.55 (2.82)
% Fraction absorbed normalized to length (%Fa/cm)	1.63 (0.07)	2.28 (0.25)	2.76 (0.3175)	3.04 (0.15)
Length required for complete absorption (L95%)	234.09 (6.22)	136.43 (25.27)	93.35 (12.22)	72.10 (2.14)
Anatomical reserve length (ARL) (cm)	-214.09 (6.22)	-116.43 (25.27)	-73.35 (12.22)	-52.10 (2.14)

 * Sodium salt at 25 mM concentration.
 ** Statistically not different from control P-value > 0.05.
 *** Statistically different from control P-value < 0.05.

permeation enhancing action on ranitidine HCl but it was statistically insignificant compared with the control. So, the enhancing action of SCFAs on the membrane transport parameters of ranitidine HCl was in the order of butyrate > propionate > acetate. In order to investigate this enhancing effect on the transport of ranitidine HCl we examined the effect of increased chain length of SCFAs on the net water flux in both segments. Figure 3 shows that the increase in the chain length of SCFAs is associated with an increase in the net water flux in both segments, especially in the ascending colon, this is because of higher water and sodium ions flux in this segment.[34] Since the major transport mechanism of ranitidine HCl across the intestinal membrane is the passive diffusion via the paracellular convective pathway as we discussed previously, thus increasing the net water flux by coperfusion of SCFAs had resulted in a significant increase in the absorptive clearance of ranitidine HCl, and all membrane transport parameters from rabbit small and large intestine. This effect was proportional to the chain length of SCFAs. The effect of butyrate on the paracellular permeability was studied on rat distal colonic epithelium using Ussing chambers.[38] The results proved that the exposure to butyrate increased paracellular permeability in rat distal colon. The mechanism involved was related to the loss of differentiated surface epithelial cells, or as a physiological response to Na^+-coupled butyrate uptake. In another study conducted on rat and rabbit distal colon,[39] butyrate significantly decreased conductance, decreased flux of sodium from serosa to mucosa, particularly in rabbit distal colon. Net Cl^- secretion, induced by cholera toxins, was completely inhibited by butyrate. The effects of SCFAs on fluid and electrolyte absorption during cholera toxin (CT) -induced colonic secretion in rabbit was studied[40] using a steady-state perfusion technique. Perfusion with SCFAs significantly ($P < 0.01$) reduced colonic water secretion. Butyrate reduced water secretion the most (95%), followed by propionate (90%), and acetate (80%). These

results are consistent with our results which proved that the enhancing effect of SCFAs on net water absorption in the rabbit colon was in the order: butyrate > propionate > acetate.
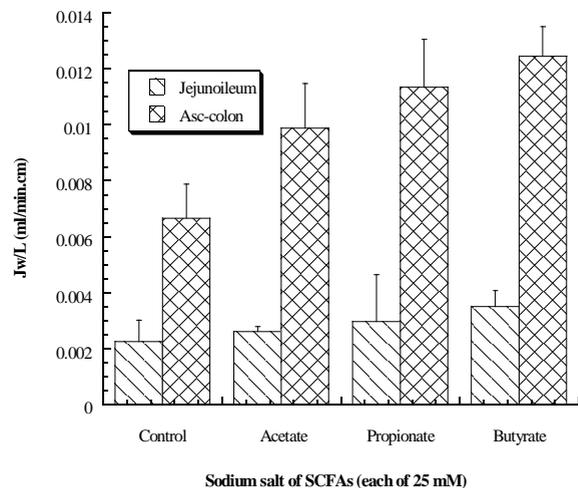
Fig. 3: Effect of SCFAs on the net water flux in the jejunoileum and ascending colon of rabit intestine (Mean + SD), (n= 3).

Conclusion

The study demonstrated that short chain fatty acids SCFAs, acetate, propionate, and butyrate had a significant effect on increasing the absorptive clearance normalized to the intestinal length (PeA/L) of ranitidine HCl in both the jejunoileum and ascending colon of the rabbit intestine. This effect was in the order butyrate > propionate > acetate, however there was no statistical difference between the effect of butyrate and propionate. The permeability enhancing effect of SCFAs was much higher in the ascending-colon, this could be attributed to the higher Na^+, Cl^-, and water influx in this segment. Marked segmental differences in the absorption of ranitidine HCl were found in the rabbit small and large intestine, the absorptive clearance (PeA/L) of this compound in the ascending colon was almost double that in the jejunoileum. Solvent drag had a significant effect on absorptive clearance (PeA/L) of ranitidine HCl which is transported across the intestinal membrane by passive diffusion via the paracellular pathway.

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