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Effect of intestinal fluid flux on ibuprofen absorption in the rat intestine

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Abstract

Previously the apparent permeability coefficient (P_{app}) of ibuprofen was observed to vary depending on the perfusion medium employed. The present work explores the possible contributions to these differences. Studies were undertaken using an in situ single pass rat gut technique. Lumenal drug concentrations and plasma drug levels were assayed by HPLC. Absorption rate constants (k_0) were determined from fractions of drug unabsorbed from the intestineat steady state. Plasma data were fitted to a two compartment open model with zero-order input. Significant differences in net fluid flux were observed between the various buffered perfusion media, with fluxes varying from -0.044 ± 0.006 ml min^{-1} to $+0.057 \pm 0.013$ ml min^{-1}, the lower and negative values occurring for lower pH media and the larger positive values tending to occur with media of higher pH. A linear relationship was found between the P_{app} of ibuprofen and net water flux ($y = 1.13 + 11.3x$; $r^2 = 0.80$). Apparent zero-order rate constants for ibuprofen appearance in plasma correlated well with absorption rate constants estimated from steady state lumenal drug concentration [$k_{0(gut)}$]. From the linear relationship between P_{app} and fluid flux a normalized P_{app} for ibuprofen (i.e. the P_{app} in the absence of net fluid flux) of 1.1×10^{-4} cm s^{-1} was determined Net lumenal fluid flux is dependent on perfusion medium composition and significantly alters ibuprofen absorption. The differences observed for P_{app} were reflected in systemic drug absorption concentrations. The findings of these studies underline the importance of standardizing the osmolarity of experimental media used for the determination of intestinal permeability data.

Keywords: Fluid flux; Ibuprofen; Osmolarity; Permeability; Intestine

1. Introduction

Secretion and absorption of fluids represent processes of major importance in the physiology of the gastrointestinal (GI) tract. The fluids that are transported across the GI barriers contain salts in water. Water flux across the intestinal epithelium occurs via both transcellular and paracellular routes but the relative magnitude of the flux through each route is not known. As in all other epithelial layers, water movement across GI epithelia can be coupled to active ionic transport or driven by an osmotic gradient (Finkelstein, 1987). The intestinal absorption of small paracellular marker molecules has been shown to increase in direct proportion to the net water absorption, suggesting that absorption could be mediated by "solvent drag" in the fluid absorbed through the paracellular pathway (Andersson and Ussing, 1957; Pappenheimer and Reiss, 1987; Krugliak et al., 1989).

Hydrophilic and charged solutes have a lower membrane permeability attributed mainly to a lower partition into the lipid membrane. The paracellular route is therefore assumed to be the major transport route accessible to these compounds in the intestine. Previously Lennernäs and co-workers have investigated the effects of water absorption in vivo in humans. For hydrophilic compounds with molecular weights between 180 and 4000 Da the jejunal P_{eff} did not increase (Lennernäs et al., 1994; Nilsson et al., 1994; Fagerholm et al., 1995).

Studies by Hirasawa et al. (1984) suggested that fluid flux had only a minor effect on the absorption of salicylic acid. However, the mechanisms underlying intestinal absorption rates and transport drugs with a monocarboxylic acid in their structure are still not fully clarified. Weak organic acids with pK_a values between 3 and 5.5 are in general rapidly absorbed from the gastrointestinal tract. The rapid absorption of these drugs is at variance with their expected low membrane permeability in the ionised form.

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Table 1
Osmolarity (mOsm) permeability coefficients (P_{app}) values, and $k_{0(gut)}$ of ibuprofen in each buffer

Buffer system	Osmolarity (mOsm)	P_{app} (cm s^{-1}) × 10^{-4}	$k_{0(plasma)}$ (mg min^{-1})
Fagerholm	286.8 ± 5.4	1.66 ± 0.17*	0.170 ± 0.061
FaSSIF	270 ± 8.1	1.17 ± 0.20	0.179 ± 0.115
FeSSIF	635 ± 7.6	0.68 ± 0.14*	0.108 ± 0.082
HBSS	310.7 ± 3.8	1.83 ± 0.11*	0.190 ± 0.094
Krebs	317.8 ± 5.7	1.72 ± 0.12*	0.186 ± 0.036
McIlvaine	415.3 ± 9.1	1.07 ± 0.14	0.167 ± 0.049
PBS 6.8	330.6 ± 6.6	1.40 ± 0.14*	0.177 ± 0.050
PBS 7.4	337.5 ± 8.2	1.30 ± 0.13	0.166 ± 0.053

* P_{app} values that are significantly different based on ANOVA ($p < 0.05$) using pair-wise comparisons as indicated in the text.

In the present study we have investigated the effects of intestinal fluid flux on the permeability of the model monocarboxylic drug ibuprofen. Linear correlation of intestinal permeability and the fraction absorbed between humans and rats has been reported for various drugs (Fagerholm et al., 1996; Chiou and Barve, 1998). Accordingly rats as an animal model appear to predict reasonably the extent of drug absorption in humans. An osmotic gradient across the intestinal epithelium stimulates movement of water and may, in parallel, modulate the absorption of compounds that are freely or partly transported with water (Hunt et al., 1992). Enhanced transepithelial water flow was therefore stimulated by using commonly employed simulated intestinal media.

2. Methods

2.1. Materials

Acetic acid (analytical grade) (Merck, Germany), calcium chloride (Riedel-de-Haen, Germany), citric acid (Merck), D-glucose (Riedel-de-Haen), ibuprofen acid (Sigma Chemical Co., Germany), magnesium sulphate (BDH Chemicals Ltd., UK), potassium acid phosphate (BDH Chemicals Ltd.), potassium chloride (BDH Chemicals Ltd.), sodium acid phosphate (Merck), sodium bicarbonate (BDH Chemicals Ltd.), sodium chloride (Merck), sodium hydroxide (Sigma), sodium phosphate (Merck) and taurocholic acid (sodium salt) (Sigma) were used in the preparation of the buffers in the quantities shown in Table 1. Lipoid E PC phosphatidylcholine (lecithin) (Lipoid GMBH, Germany) was a gift from the manufacturer and was used as received.

2.2. Composition of buffer systems

The buffer systems examined were: Sorensens phosphate buffer pH 7.4 (Wade, 1980), sodium phosphate perfusion solution (Fagerholm et al., 1996), McIlvaine buffer (Xiang et al., 2002) pH 6.0, HBSS (Crowe and Lemaire, 1998), Krebs buffer (Leone-Bay et al., 1996; Lund, 1994), Sorensens phosphate buffer pH 6.8 (Wade, 1980), FaSSIF (Galia et al., 1998), FeSSIF (Galia et al., 1998). The compositions of Fagerholm's (Fagerholm et al., 1996), McIlvaine's (Pharm. Handbook, 1980) and Krebs' (Pharm. Codex, 1994) buffers and Hank's balanced salt solution (HBSS) have been reported previously (Levis et al., 2003).

2.3. Osmolarity

Osmolarity was determined from the molar concentrations of buffer components and confirmed by the vapour pressure method using an osmometer (Fiske Associates, Norwood, MA).

2.4. In situ absorption studies

In situ absorption studies were carried out on male Wistar rats (280–320 g) that had been fasted for 24 h prior to the experiment and were anaesthetised by intraperitoneal (i.p.) injection of pentobarbital sodium (50 mg kg^{-1}). The studies were conducted according to the rat gut perfusion method described by Komiya et al. (1980) using a 33.3 cm length of intestine and a flow rate of 0.2 ml min^{-1}. Perfusate samples were collected every 10 min for a period of 120 min and were assayed for drug content by HPLC. Blood samples were taken at 30-min intervals, centrifuged to separate the plasma and the plasma was then frozen until analysis. The intestines were kept moist throughout the experiment by gently applying buffer using cotton wool balls saturated with warm saline and body temperature was maintained at 37 °C using an overhead work-light and a heating mat. Sample vials were weighed prior to use and after perfusate collection in order to check the flow rate and to determine any variation in the volume of liquid collected. Perfusate samples were filtered and analysed by HPLC using a method based on that used by Lalande et al. (1986).

The fraction of ibuprofen unabsorbed was calculated for each perfusate sample and at each time point for a particular buffer these values were averaged over the number of rats studied ($n \geq 5$). Fraction of ibuprofen unabsorbed against time profiles were generated for all buffers and converted to permeability coefficients using steady-state data and Eq. (1).

$$P_{app} = \frac{-Q}{2\pi r l} \ln\left(\frac{C_1}{C_0}\right) \quad (1)$$

where C_0 is the input perfusate drug concentration, C_1 is the outlet perfusate drug concentration, r is the effective lumen radius (cm), Q is the perfusate flow rate (ml s^{-1}), and l is the length of intestinal segment (33.3 cm). The permeability coefficient

(P_{app}) for ibuprofen was obtained by averaging the permeability coefficients over 100–120 min for each perfusion experiment. The P_{app} value reflects contributions for ionized and unionized species. For a particular buffer, the P_{app} values for each rat were averaged and a standard deviation calculated. Pair-wise comparisons were performed using a one-way ANOVA using Minitab™ Statistical Software (version 13.1).

2.5. pH

The pH of all buffers and perfusate samples was measured using an Orion small volume electrode connected to an Orion 250A pH meter.

2.6. Calculation of fluid flux values

The method described by Sutton and Rinaldi (2001) and Peréz et al. (2002) was used (Eq. (2)) and assumes that perfusate sample weight is equal to its volume.

$$C_{l\prime} = C_l \left(\frac{W_{out}}{W_{in}} \right) \quad (2)$$

where W_{out} is average sample weight, W_{in} is 2.0 g (as input flow rate is 0.2 ml min^{-1} with samples collected over 10 min), C_l is the concentration of compound in the sample.

2.7. Plasma analysis

Plasma was separated by centrifugation at 5000 × g at 4 °C for 15 min using a Sorvall refrigerated centrifuge. The supernatant was pipetted into an HPLC autosampler vial without dilution and analysed for drug and internal standard by the same HPLC method as for the perfusate samples.

2.8. Pharmacokinetic modelling of intestinal and plasma data

An input rate constant for ibuprofen absorption from the intestine is calculated for each buffer system at the steady-state, $k_{0(gut)}$ using Eq. (3):

$$k_{0(gut)} = \frac{\pi r^2 l (C_0 - C_l)}{t} \quad (3)$$

where r is the effective lumen radius (0.18 cm); l is the length of intestinal segment (33.3 cm); C_0 is the input perfusate drug concentration; C_l is the outlet perfusate drug concentration at the steady-state; t is the time taken for a drug molecule to pass through the length of intestinal segment.

A two compartment model with constant input [$k_{0(plasma)}$] and first-order output was fitted to the plasma data using average best-fit parameters (Scientist®). The parameters in this model are k_{elim} (elimination rate constant), k_{12} (rate constant for transfer from the central to the peripheral compartment), k_{21} (rate constant for transfer from the peripheral to the central compartment) and volume of distribution (V).

3. Results

3.1. Ibuprofen permeability coefficients

The permeability coefficient (P_{app}) values calculated from in situ absorption studies are reported for the eight buffers of differing composition in Table 1. For the Fagerholm, HBSS and Krebs buffer systems the P_{app} values are significantly higher than FaSSIF, FeSSIF, McIlvaine, PBS 6.8 and PBS 7.4. PBS 6.8 is significantly higher than McIlvaine and FeSSIF. FeSSIF is significantly lower than the other seven buffers ($p < 0.05$).

3.2. Influence of pH

pH values of the perfusate samples were monitored over the course of each experiment (Fig. 1). The buffers studied varied in pH over a range of 3.21 pH units from 5.01 to 8.22. With the exception of the FeSSIF system, ibuprofen is 98–99.9% ionised in the buffer systems studied. Over the time course of each perfusion experiment, the pH values of the samples gradually change from their initial values towards a median value of approximately 6.5, with some of the systems being shifted upwards and others shifted downwards from their initial values.

The magnitude of these pH changes is significant for four of the buffers: FaSSIF, FeSSIF, HBSS and PBS 7.4 ($p < 0.05$). This would be expected as FaSSIF, HBSS, PBS 7.4 and Krebs' correspond to the four lowest buffer capacity values of the perfusion solutions as reported previously (Levis et al., 2003). Despite its high buffer capacity, FeSSIF shows a significant change in pH over the course of the perfusion. This may be because of its relatively low pH (compared to the microclimate pH) that may stimulate the intrinsic intestinal buffering system to produce the pH change observed. In the case of most of the buffers, the change in pH occurs over ∼50 min after which there is little change. Desai (1977) reported that the pH of buffered solutions, initially pH 9.5 and 4.5, respectively, of low buffer capacity tended rapidly towards a pH of 6.5 when placed in the rat intestine and Ikuma et al. (1996) have shown that the jejunal microclimate pH in young adult rats is 6.12 ± 0.04.

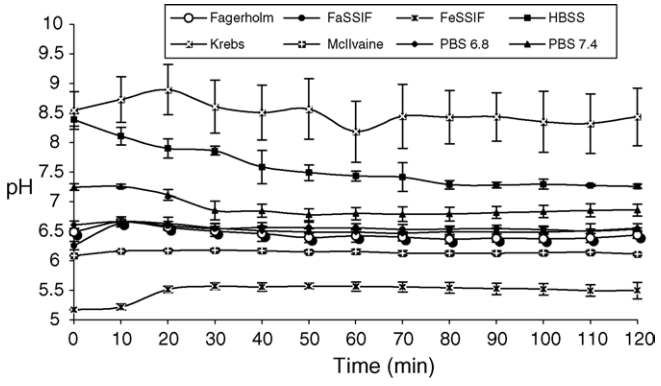

Fig. 1. pH vs. time profiles of perfusate samples.

The changes in the pH values of the perfusate samples will affect the percentage of drug ionised. Despite some of the pH changes being significant, the corresponding change in fraction of ibuprofen ionised is negligible for all buffers except FeSSIF. The initial pH of the FeSSIF perfusion solution (5.17) corresponds to 84.6% ionised and its pH at the steady-state (5.51) corresponds to 92.3% ionised. At the steady-state, the remaining seven buffers give 98–99.9% of drug ionised. According to the pH-partition hypothesis there is therefore a greater fraction of unionised ibuprofen available to the lipid membrane from FeSSIF than for the other buffer systems. However, this does not result in enhanced ibuprofen permeability for the compound, as its P_{app} value is significantly lower than the P_{app} values for Fagerhom, Krebs, HBSS and PBS 6.8.

3.3. Buffer osmolarity

The osmolarity values for all buffer systems are presented in Table 1. Osmolarities of six of the eight buffers lie within, or close to, the physiological range (280–320 mosmol l^{-1}), exceptions being FeSSIF and McIlvaine buffers, which are hyperosmotic. As well as having the highest osmolarities, these two buffers also show the lowest ibuprofen P_{app} values (Table 2). The ibuprofen P_{app} in FeSSIF (0.68×10^{-4} cm s^{-1}) is significantly lower than for all the other buffers. The P_{app} in McIlvaine (1.07×10^{-4} cm s^{-1}) is the second lowest and is significantly lower than four of the other buffers.

The relationship between buffer osmolarity and ibuprofen P_{app} is shown in Fig. 2. As osmolarity increases, there is an apparent decrease in ibuprofen permeability. Conversely, decreased osmolarity results in enhanced ibuprofen permeability. Hypotonic or glucose-rich solutions have increased the intestinal absorption of some but not all drugs in studies carried out in experimental animals and humans as reviewed by Lennernäs (1995), and in vitro in isolated intestinal tissues and cell monolayers (Atisook and Madara, 1991; Fricker and Drewe, 1995).

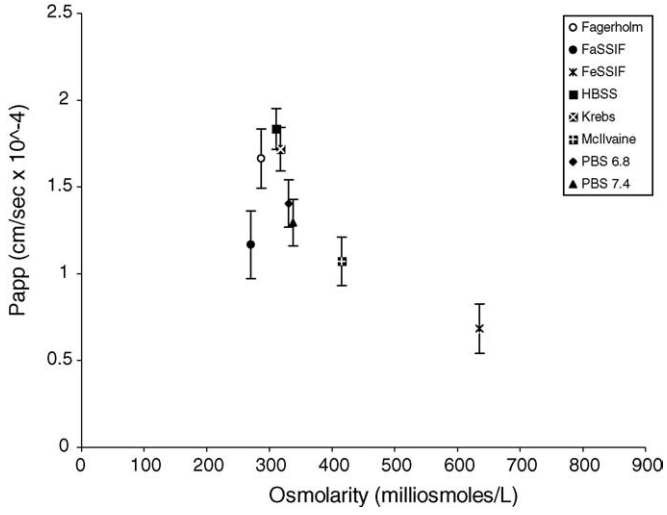

Fig. 2. Relationship between P_{app} and buffer osmolarity.

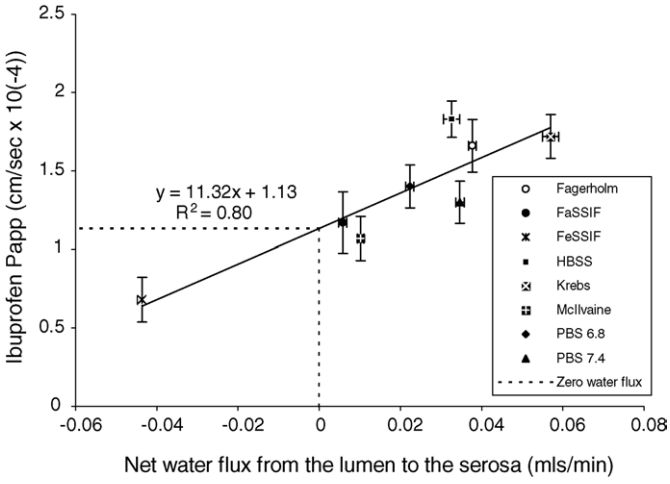

Fig. 3. Relationship between ibuprofen P_{app} and net water flux.

3.4. Fluid flux

Fig. 3 illustrates the relationship between ibuprofen P_{app} and net water flux across the intestinal wall from the lumen to the serosa. A negative value for net water flux represents secretion into the lumen and a positive value represents absorption

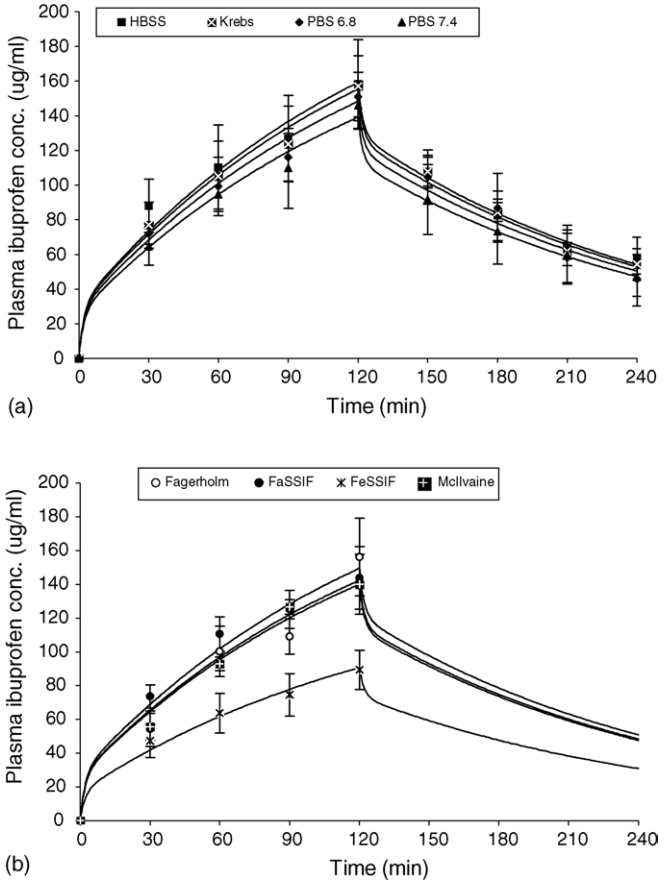

Fig. 4. (a) The best-fit plasma ibuprofen concentration vs. time profiles together with the experimental data points for HBSS, Krebs', PBS 6.8 and PBS 7.4. (b) The best-fit plasma ibuprofen concentration versus time profiles together with the experimental data points for Fagerholm's, FaSSIF, FeSSIF and McIlvaine's.

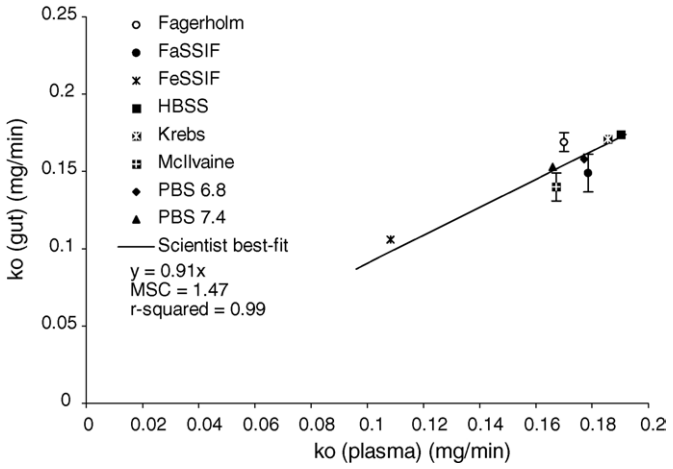

Fig. 5. Relationship between $k_{0(gut)}$ and $k_{0(plasma)}$.

from the lumen. As water flux increases from the mucosal to the serosal side it increases P_{app} and, conversely, as it increases from the serosal to the mucosal side it reduces P_{app}. The hyperosmotic buffers, FeSSIF may be generating reduced estimates of ibuprofen P_{app} by producing changes in water secretion into the intestine relative to the other buffers. Extrapolating a line from zero water flux up to the line of best-fit and to P_{app} on the y-axis generates a value for ibuprofen P_{app} when there is no water flux. This value is 1.13×10^{-4} cm s^{-1}. Hypotonic solutions have previously been observed to induce increased net water absorption in both humans and rats (Fagerholm et al., 1999); hypotonic solutions drive water absorption and hypertonic solutions hinder it.

3.5. Pharmacokinetic modeling of intestinal steady state data and plasma profiles

The pharmacokinetics of ibuprofen in the rat have previously been described by a two compartment model with first order output (Parrott and Christensen, 1984; Shah and Jung, 1987; Itoh et al., 1997). The plasma ibuprofen concentration versus time profiles fitted to the model for all eight systems are shown in Fig. 4. The zero-order input rate constant determined from plasma, $k_{0(plasma)}$ together with the zero-order intestinal input rate constant, $k_{0(gut)}$ from Eq. (3) are plotted against each other in Fig. 5. The rate of appearance of ibuprofen in the plasma [$k_{0(plasma)}$] is proportional to the rate of intestinal absorption [$k_{0(gut)}$] at the steady-state.

4. Discussion

Oral drug absorption studies in the literature have employed a variety of in vitro and in vivo models including Ussing chambers, everted gut sac techniques, cell culture models, in situ perfusions and intestinal perfusions in man (Stewart et al., 1997). In parallel with these different models, researchers have employed a variety of simulated intestinal media or buffer solutions which are diverse in composition and osmolarity. These range from simple phosphate based systems (PBS) at pH 7.4 (Iwanaga et al., 1999) to more complex systems containing lipids and surfactants to simulate intestinal contents, i.e., the fasted (FaSSIF) and fed (FeSSIF) state simulated intestinal fluids (Galia et al., 1998).

The present study demonstrates that water flux significantly affects the permeability coefficient of ibuprofen. Each system caused a certain amount of water flux across the intestinal mucosa, which influenced the P_{app} values. Hyperosmolarity was clearly accompanied by a decrease in permeability probably caused by reversed solvent drag. From a plot of P_{app} against net water flux, the P_{app} of ibuprofen in the absence of water flux was calculated as 1.13×10^{-4} cm s^{-1}. At the pH values of the buffer systems used in the present study ibuprofen is expected to be 98–99% ionized for all vehicles except FeSSIF in which it is >90% ionized. Thus, where buffer osmolarity might be expected to have a significant effect on drug permeability, care should be taken to standardise the osmolarity of the particular buffer used to simulate the intestinal fluid. Comparisons of permability data obtained using different models and different media may otherwise not be valid.

The intestinal absorption of small marker compounds and water during solvent drag has been assumed to occur through the paracellular route to a considerable extent (>50%) (Armstrong, 1987; Madara and Trier, 1994). However, transport routes of water and hydrophilic compounds are not fully clarified (Bjarnason et al., 1995; Lane et al., 1996). A transcellular pathway for the movement of water in the small intestine has been suggested where half of the water uptake occurs isotonically by the action of cotransporters such as the SGLT1 Na-glucose cotransporter and the other half is driven by an osmotic gradient and may involve specialized water transporter proteins termed “aquaporins” (Loo et al., 1996; Zeuthen et al., 2001).

The reported effects of fluid flux on drug permeability have been observed to vary depending on the size, charge and lipophilicity of the compound being investigated. Solvent drag was not observed to affect human permeability of marker compounds with a radius and molecular weight over 4.0 Å and 180 Da, respectively (Lennernäs et al., 1994; Nilsson et al., 1994; Lennernäs, 1995; Fagerholm et al., 1995). The permeability of small hydrophilic compounds such as urea (MW 60, molecular radius 2.6 Å) and creatinine (MW 113, molecular size 7.2 × 8.0 Å) in humans was affected by solvent drag (Fagerholm et al., 1999). In a further study by Fagerholm et al. (1999) increased permeability of antipyrine (MW 188), a marker of passive transcellular transport was observed in the rat after induction of net fluid absorption with hypotonic glucose solution. This was ascribed to a higher concentration gradient of the drug close to the intestinal wall, and thereby increased transcellular absorption.

The secretion and reabsorption of water will modify the luminal concentration of ibuprofen and therefore its rate of absorption. This allows for the possibility that for drugs which are absorbed transcellularly, the solvent drag effect may not be attributed solely to increased paracellular flux. Increased solute concentration close to the intestinal wall and/or a direct effect

of water absorption on the cell membrane may also contribute to permeability changes.

The in situ model used in the present study has the advantage of allowing sampling from the jugular vein, with the rate of ibuprofen appearance in plasma mirroring the trends observed for the intestinal permeability values. A good correlation was observed for apparent zero-order plasma input rate constants (k_0) and intestinal absorption rate constants estimated from steady state lumenal drug concentration. This supports the hypothesis that the differences observed for P_{app} were not artefacts of the methodology and reflect systemic drug absorption. Net lumenal fluid flux is dependent on perfusion medium composition and significantly alters ibuprofen absorption.

5. Conclusions

In the present study, solvent drag-induced ibuprofen transport in the rat small intestine was demonstrated and had a significant effect on ibuprofen permeability. Standardisation of the osmolarity of experimental media simulating the small intestinal fluid will be of practical importance for permeability determinations of ibuprofen in vitro and in situ. A linear relationship between the rate of drug disappearance from the intestinal lumen and drug appearance in plasma is observed for the in situ model employed in this work.

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