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Polarised transport of monocarboxylic acid type drugs across rat jejunum in vitro: the effect of mucolysis and ATP-depletion

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Abstract

The transport characteristics of monocarboxylic acid type drugs (ketoprofen, ibuprofen and gemfibrozil) across the excised jejunal segments and artificial (octanol impregnated) membrane in side-by-side diffusion cells were studied. All three model drugs permeated faster across the intestinal tissue in the mucosal-to-serosal direction than in the opposite direction. No polarised transport of tested drugs was observed when the mucosal side of the intestine was treated with mucus disrupting agent, L-cysteine 1% (w/v), which significantly increased the microclimate pH at the mucosal surface of the intestine. Similar effects on the transport characteristics of model drugs and microclimate pH were observed when metabolic inhibitor, sodium azide (10 mM), was present in the incubation medium. Furthermore, the direction of proton gradient across the artificial membrane was shown to significantly influence the transport of model drugs across this membrane. The results of this study indicate that the inwardly directed proton gradient maintained by the acidic microclimate pH at the intestinal surface could be considered as a driving force for the transport of monocarboxylic acid type drugs across the intestinal epithelia and could explain rapid absorption of these drugs after oral application.

Keywords: Monocarboxylic acid type drugs; Artificial membrane; Microclimate pH; ATP-depletion; Mucolysis; Rat jejunum

1. Introduction

Many drugs have monocarboxylic group in their structure. These weak organic acids with pK_a values between 3 and 5.5 are in general rapidly absorbed from the gastrointestinal tract, however, the mechanism of transport across the intestinal epithelia is still uncertain. The pH-partition hypothesis predicts that weak electrolytes are absorbed from the gastrointestinal tract by passive diffusion depending on the fraction of undissociated organic acid at the pH on the mucosal surface of the intestine (Brodie and Hogben, 1957; Högerle and Winne, 1983).

It is difficult to reconcile rapid absorption of monocarboxylic acid type drugs with this concept because these substances are almost completely ionised at the pH on the mucosal surface of the intestine (pH 6.0–7.1; Rechkemmer et al., 1986; Shimada, 1987; Ikuma et al., 1996).

A mechanism which could explain rapid absorption of monocarboxylic acids has been suggested by Takagi et al. (1998), based on the uptake studies of salicylic acid in artificial liposomes. They have suggested that the absorption of monocarboxylic acid type drugs could be facilitated by the inwardly directed proton gradient across the brush border membrane, which is

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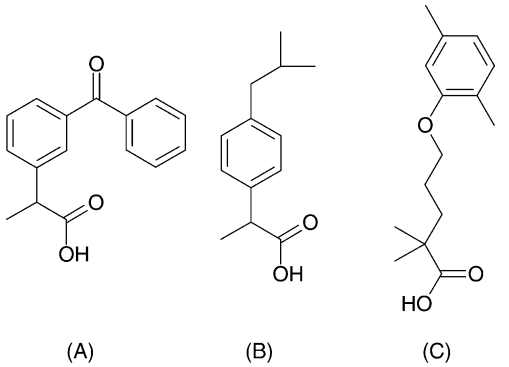

Fig. 1. Structures of (A) ketoprofen, (B) ibuprofen and (C) gemfibrozil.

maintained by the acidic microclimate on the mucosal surface. However, no direct evaluation of this mechanism has been undertaken in vivo or in an appropriate in vitro system.

The aim of the present study was to evaluate the effect of microclimate pH on the transport characteristics of three model monocarboxylic acid type drugs; two non-steroidal anti-inflammatory drugs (NSAIDs): ketoprofen (Fig. 1A) and ibuprofen (Fig. 1B) and a hypolipidemic drug, gemfibrozil (Fig. 1C). Transport experiments across the excised segments of rat jejunum were performed in side-by-side diffusion chambers. Microclimate pH was measured with pH microelectrodes. Additionally, the transport of these model monocarboxylic acid type drugs across the artificial membrane mounted in side-by-side diffusion chambers under different conditions regarding pH gradient was also examined.

2. Materials and methods

2.1. Materials

Ketoprofen, ibuprofen, gemfibrozil and L-cysteine were from Sigma Aldrich Chemie (Deisenhofen, Germany). Fluorescein sodium was purchased from Fluka (Deisenhofen, Germany). Sodium azide was obtained from Riedel-de Haën AG Seelze (Hannover, Germany). All chemicals used in this study were of analytical grade.

2.2. Transport studies across rat jejunum in vitro

The experiments conform to the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (Council of Europe No. 123, Strasbourg, 1986).

Rat jejunum was obtained from male Wistar rats (250–320 g). All animals were starved for 18 h before the experiments. After decapitation and laparotomy, the small intestine was immediately excised and placed into the ice-cold bubbled (carbogen, 95:5 O_2/CO_2) 10 mM solution of D-glucose in standard Ringer buffer containing (in mM): 140.6 Na^+, 5 K^+, 1.2 Ca^{2+}, 1.2 Mg^{2+}, 121.8 Cl^-, 25 HCO_3^-, 0.4 $H_2PO_4^-$, 1.6 HPO_4^{2-}. Jejunum 25 cm distally from the pyloric sphincter was used in the experiments. The tissue was rinsed with ice-cold standard Ringer buffer to remove luminal content and cut into 3 cm long segments, excluding visible Peyer's patches. The intestinal segments were opened along the mesenteric border, stretched onto a special insert and placed between the two EasyMount side-by-side diffusion chambers with the exposed tissue area of 1 cm^2 (Physiologic Instruments, San Diego, CA, USA).

During the experiments the intestinal segments were bathed on both sides with standard Ringer buffer (pH 7.5) supplemented with 10 mM D-glucose at the serosal and 10 mM mannitol at the mucosal side. The bathing solution was continuously gassed with carbogen (95:5 O_2/CO_2) and kept at 37 °C.

After preincubation for 25 min, the investigated monocarboxylic acid was added to the mucosal or serosal side if studying mucosal-to-serosal (m-to-s) or serosal-to-mucosal (s-to-m) transport, respectively. The final volume of the solution in each compartment was 2.5 ml. The concentration of the investigated substances in the donor compartment was 250 μM.

Samples of 250 μl were withdrawn from the acceptor compartment at 25-min intervals up to 175 min and replaced with fresh Ringer buffer containing 10 mM D-glucose (m-to-s transport) or 10 mM mannitol (s-to-m transport) to maintain a constant volume. Effect of compound withdrawal was taken into account for when calculating apparent permeability coefficient (P_{app}) values.

The diffusion chambers were equipped with two pairs of Ag/AgCl electrodes connected to the chambers via 3 M KCl/3.5% agar bridges, for measuring

transepithelial potential difference (PD) and for passing current, respectively. The experiments were performed under the short-circuited conditions; the current was delivered by a multichannel voltage–current clamp (model VCC MC6, Physiologic Instruments). The tissue viability and integrity were checked by monitoring PD, short circuit current (I_{sc}) and tissue electrical resistance (TER) every 25 min and additionally by recording the increase of I_{sc} and PD after the addition of D-glucose (25 mM) to the mucosal compartment at the end of experiments. TER was determined according to the Ohm's law. I_{sc} and TER were corrected for fluid resistance before mounting the tissue in the diffusion chamber system.

The tissue integrity during transport studies was also validated by measuring the permeability of a hydrophilic transport marker, fluorescein sodium, with a donor concentration of 5 μM.

2.3. In vitro measurements of mucosal surface pH

The pH measurements were performed with a flat membrane pH microelectrode (MI-406, tip diameter 1.5 mm, Microelectrodes, Inc., Bedford, NH). The reference electrode, MI-402 (Microelectrodes, Inc.) was dipped into the same incubation medium as the pH microelectrode. Both electrodes were connected to a digital pH meter (model MA-5736, Iskra, Ljubljana, Slovenia). The tissue was first incubated in the diffusion chambers under the same conditions as applied in the transport experiments. The insert with the mounted tissue was then placed into a thermostated bath (37 °C) with the mucosal surface upwards. The flow (4 ml/min) through the bath was maintained with the same solution as used in the diffusion chambers. This solution was kept in a reservoir at 37 °C and gassed with carbogen. After pH determination of the bathing solution, the pH microelectrode was advanced to the epithelial surface by using a micromanipulator until the tip of the electrode touched the mucus layer; this was noticed as a change in pH. Afterwards the electrode was lowered down for additional 0.5 mm. Stable pH reading was achieved within 3–4 min.

The procedure of the pH determination did not affect the tissue viability. This was checked by monitoring the electrical parameters of the tissue before and after pH measurements.

2.4. Transport studies across the artificial membrane

Permeability across the artificial lipid membrane was studied in Sweetana–Grass type diffusion chambers custom made at the University of Utrecht, The Netherlands (the volume of donor and acceptor solution is 2.5 ml, exposed surface area is 1.22 cm^2). The artificial lipid barrier was prepared from a cellulose nitrate membrane filter with 0.1 μm pore size (Sartorius, Göttingen, Germany), which was impregnated with 1-octanol for 1 day; the excess of octanol was removed by pressing the impregnated membrane filter between the two pieces of blotting paper. The impregnated filter disc was then cut into six pieces, which were mounted in the diffusion chambers. Afterwards, the membrane inside the diffusion chambers was exposed to the same buffers as used later in the experiment for 90 min to ensure that the lipid phase was saturated with aqueous phase. Experiments were performed with Ringer buffer solutions, which were modified in some cases to obtain the appropriate pH. The experimental conditions were: pH 6.5 on donor and pH 7.5 on acceptor side, pH 7.5 on both sides, pH 7.5 on donor and pH 6.5 on acceptor side. The concentration of ketoprofen, ibuprofen and gemfibrozil in the donor compartment was 500 μM. Samples of 250 μl were removed from the acceptor compartment at 10-min intervals up to 60 min and replaced with the equal volume of the appropriate Ringer buffer.

2.5. Analytical procedure

The concentrations of ketoprofen, ibuprofen, gemfibrozil and fluorescein in the samples from the transport experiments were analysed with HPLC system (Series 1100, Hewlett Packard, Waldbron, Germany). The column Eurospher C-8 (5 μm, 250 mm × 4 mm; Bia Separations, Ljubljana, Slovenia) was used at 35 °C. The mobile phase for all studied substances was composed from acetonitrile and phosphate buffer (pH 7.5) in different ratios; 15:85 for ketoprofen and fluorescein, 23.5:76.5 for ibuprofen and 28.5:71.5 for gemfibrozil. The detection was by UV absorption using diode array detector at 262 nm for ketoprofen and 225 nm for ibuprofen and gemfibrozil. Fluorescein was detected by fluorescence detector (λ_{EX} = 487 nm, λ_{EM} = 510 nm) (model RF-535, Shimadzu, Kyoto, Japan).

2.6. Data analysis and statistics

The P_{app} value of the investigated substances was calculated from the following equation:

$$P_{app} = \frac{dQ}{dt}\frac{1}{AC_0}\ (cm/s) \quad (1)$$

where dQ/dt is the steady-state appearance rate on the acceptor side of the tissue or artificial membrane, A is the exposed area of the tissue (1 cm^2) or artificial membrane (1.22 cm^2), C_0 is the initial concentration of the drug in the donor compartment.

All data are presented as means ± S.E.M. Statistical significance was evaluated by two-tailed Student's *t*-test or ANOVA.

3. Results and discussion

The effect of microclimate pH at the mucosal surface of rat jejunum on the m-to-s and s-to-m transport of three model monocarboxylic acid type drugs [ketoprofen ($pK_a = 4.6$; Fagerholm et al., 1996), gemfibrozil ($pK_a = 5$; Ungell et al., 1998) and ibuprofen ($pK_a = 5.3$; Beetge et al., 2000)] across rat jejunum in vitro was examined. Polarised transport of all three investigated substances was observed when both sides of the tissue were bathed with standard Ringer buffer (pH 7.5). The transport of the investigated substances in the m-to-s direction significantly exceeds the transport in the opposite direction (control, Table 1). Under these conditions (control), an acidic microclimate layer on the mucosal surface was detected with pH 6.99 ± 0.03 ($n = 10$), which is significantly lower ($P < 0.0001$, *t*-test) than the incubation medium pH 7.51 ± 0.02 ($n = 10$).

Destruction or solubilisation of mucus by S–S reducing agents, such as 1,4-dithio-DL-threitol, L-cysteine and *N*-acetyl-L-cysteine, is known to affect microclimate pH because the role of mucus being a barrier to H^+ movement is deteriorated (Shimada, 1987; Ikuma et al., 1996). When the mucosal side of the tissue was treated with L-cysteine, the microclimate pH increased (compared to the control experiment) significantly ($P < 0.0001$, *t*-test) to 7.28 ± 0.02 ($n = 6$), while the asymmetry in the transport of the investigated substances across the intestine was abolished (Table 1). Similar effects on the microclimate pH and transport characteristics of the investigated substances were observed when metabolic inhibitor sodium azide was present in the incubation medium. The acidity of the microclimate layer was reduced significantly ($P < 0.0001$, *t*-test) to 7.33 ± 0.02 ($n = 6$). At the same time no significant differences between the P_{app} values estimated for m-to-s and s-to-m transports were observed (Table 1). Similar effects of metabolic inhibitors (iodoacetate and dinitrophenol) on the microclimate pH were observed by Said et al. (1987).

These results demonstrate the importance of inwardly directed proton gradient across the brush border membrane in the transport of monocarboxylic acid type drugs across the intestinal epithelia. Inwardly directed proton gradient across the apical membrane is maintained because of the differences between the microclimate pH at the mucosal surface (~7.0) and the intracellular pH (~7.2; Wakabayashi et al., 1997). Under these conditions, the lipophilic non-ionised form of monocarboxylic acid is quickly transported across the lipoidal membrane, followed by a redissociation in the intracellular compartment. High concentration gradient of the non-ionised form of organic acid is thus maintained across the brush border membrane, which facilitates the transport of organic acids in the m-to-s direction. At the same time, the gradient of the non-ionised form of organic acid across the apical membrane in the s-to-m direction is reduced, which hinders the transport of organic acids in this direction. This mechanism predicts that the transport of monocarboxylic acids across the apical membrane is the rate limiting factor in the absorption of these compounds from gastrointestinal tract.

One could expect no or very low proton gradient across the brush border membrane in the case of L-cysteine or sodium azide treatment because the microclimate pH increased significantly (from 6.99 to 7.28 and 7.33 for L-cysteine and sodium azide, respectively). In addition, sodium azide as an ATP-depleting agent affects the regulation of intracellular pH (Alberts et al., 1994; Hayashi and Suzuki, 1998), which further prevents the formation of the inwardly directed proton gradient across the apical membrane. Consequently, there exists no driving force for the m-to-s and no hindering force for the s-to-m transport of the investigated substances across the apical membrane, which leads to the non-polarised transport (Table 1).

Table 1
The effect of L-cysteine and sodium azide on the transport of model monocarboxylic acid type drugs (ketoprofen, ibuprofen and gemfibrozil) across rat jejunum in vitro[a]

Investigated substance	Conditions	Apparent permeability, P_{app} (10^{-5} cm/s)		$P_{app}^{m-to-s}/P_{app}^{s-to-m}$
		m-to-s	s-to-m	
Ketoprofen	Control	1.91 ± 0.14*	1.14 ± 0.14	1.68 ± 0.38
	L-Cysteine[b]	1.89 ± 0.06	2.08 ± 0.19	0.91 ± 0.08
	Sodium azide[c]	1.72 ± 0.09	1.65 ± 0.03	1.04 ± 0.07
Ibuprofen	Control	3.21 ± 0.12*	2.00 ± 0.04	1.60 ± 0.09
	L-Cysteine[b]	3.05 ± 0.22	2.54 ± 0.09	1.20 ± 0.13
	Sodium azide[c]	2.86 ± 0.25	2.51 ± 0.13	1.14 ± 0.17
Gemfibrozil	Control	2.77 ± 0.14*	1.86 ± 0.21	1.49 ± 0.27
	L-Cysteine[b]	2.78 ± 0.20	2.31 ± 0.11	1.20 ± 0.15
	Sodium azide[c]	3.27 ± 0.18	3.35 ± 0.03	0.98 ± 0.06

[a] The incubation medium pH was 7.51 on both sides of the tissue. The P_{app} values were estimated for the mucosal-to-serosal (m-to-s) and serosal-to-mucosal (s-to-m) transport. Data are presented as means ± S.E.M. of three to six determinations. The donor concentration of the monocarboxylic acid was 250 μM.
[b] Mucosal side was treated for 15 min with 1% (w/v) L-cysteine (mucus disrupting agent) before the addition of investigated substance to the donor compartment.
[c] Sodium azide (10 mM, metabolic inhibitor) was present in the incubation medium on both sides of the tissue throughout the experiments.
* Significantly different from the corresponding P_{app} value estimated for the s-to-m transport ($P < 0.05$, *t*-test).

One can see that the s-to-m permeability increased pronouncedly, while the m-to-s permeability did not decrease (Table 1). This could be explained by the fact that L-cysteine and sodium azide do not selectively affect the microclimate pH, but have also other effects on the intestinal tissue. Firstly, L-cysteine reduces viscoelasticity of the mucus by disruption of disulphide linkages between glycoprotein sub-units (Poelma et al., 1990; Khanvilkar et al., 2001). Mucus layer could represent a significant barrier to the absorption of various substances (Wikman Larhed et al., 1998; Legen and Kristl, 2001; Khanvilkar et al., 2001). The destruction of the mucus structure could thus lead to the increased transport of model monocarboxylic acids across the intestinal tissue in both directions. Secondly, sodium azide in the incubation medium caused a rapid decrease of TER values during the experiments, which was associated with the significantly ($P < 0.0001$, *t*-test) increased transport of a paracellular transport marker fluorescein in both directions (P_{app} values: $6.00 \times 10^{-6} \pm 0.52 \times 10^{-6}$ cm/s ($n = 9$), $6.74 \times 10^{-6} \pm 0.51 \times 10^{-6}$ cm/s ($n = 9$) for m-to-s and s-to-m transport under the control conditions, respectively, and $9.83 \times 10^{-6} \pm 0.39 \times 10^{-6}$ cm/s ($n = 12$), $11.4 \times 10^{-6} \pm 0.7 \times 10^{-6}$ cm/s ($n = 9$) for m-to-s and s-to-m transport under the ATP-depleted conditions, respectively). Similar increase in paracellular permeability after ATP-depletion was observed by other authors (Mandel et al., 1993). These effects of L-cysteine and sodium azide could thus modify the effect of the abolishment of proton gradient across the brush border membrane on the transport of model drugs across the intestinal tissue.

To evaluate the effect of proton gradient across a simple lipid membrane, we studied the transport of investigated substances across the octanol-impregnated artificial membrane, which could represent the apical membrane of the enterocytes. Table 2 shows that the transport of all three model monocarboxylic acid type drugs across the artificial membrane was the highest in the direction of increasing pH (pH 6.5 → pH 7.5), and the lowest in the direction of decreasing pH (pH 7.5 → pH 6.5). These results demonstrate the importance of proton gradient across the lipid membrane in the transport of weak organic acids and support the suggested mechanism of the transport of monocarboxylic acids across the excised intestinal segments.

In summary, we have demonstrated the importance of low microclimate pH at the mucosal surface and normal intracellular metabolism in the transport of three model monocarboxylic acid type drugs across rat jejunum in vitro. The results from this study suggest

Table 2
The effect of proton gradient on the transport of model monocarboxylic acid type drugs (ketoprofen, ibuprofen and gemfibrozil) across the artificial lipid membrane[a]

Investigated substance	Apparent permeability, P_{app} (10^{-5} cm/s)		
	pH 6.5 → pH 7.5	pH 7.5 → pH 7.5	pH 7.5 → pH 6.5
Ketoprofen	10.3 ± 1.1	2.90 ± 0.28	2.50 ± 0.20
Ibuprofen	38.7 ± 1.3	17.5 ± 0.7	7.40 ± 0.22
Gemfibrozil	19.1 ± 1.0	14.6 ± 0.9	3.59 ± 0.14

For each model monocarboxylic acid a significant influence of experimental conditions (pH gradient) on apparent permeability (P_{app}) was found ($P < 0.01$, ANOVA).

[a] Data are presented as means ± S.E.M. of four determinations. The donor concentration of monocarboxylic acid was 500 μM.

that the inwardly directed proton gradient across the apical membrane of the enterocytes could represent the driving force for the transport of weak organic acids across the intestinal epithelia. This mechanism might explain rapid absorption of monocarboxylic acid type drugs from gastrointestinal tract.

References

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., Watson, J.D., 1994. Molecular Biology of the Cell, 3rd ed. Garland Publishing, Inc., New York and London.

Beetge, E., du Plessis, J., Müller, G.D., Goosen, C., van Rensburg, F.J., 2000. The influence of the physicochemical characteristics and phamacokinetic properties of selected NSAID's on their transdermal absorption. Int. J. Pharm. 193, 261–264.

Brodie, B.B., Hogben, C.A.M., 1957. Some physico-chemical factors in drug action. J. Pharm. Pharmacol. 9, 345–380.

Fagerholm, U., Johansson, M., Lennernäs, H., 1996. Comparison between permeability coefficients in rat and human jejunum. Pharm. Res. 13, 1336–1341.

Hayashi, H., Suzuki, Y., 1998. Regulation of intracellular pH during H^+-coupled oligopeptide absorption in enterocytes from guinea-pig ileum. J. Physiol. 511, 573–586.

Högerle, M.L., Winne, D., 1983. Drug absorption by the rat jejunum perfused in situ. Naunyn Schmiedebergs Arch. Pharmacol. 322, 249–255.

Ikuma, M., Hanai, H., Kaneko, E., Hayashi, H., Hoshi, T., 1996. Effects of aging on the microclimate pH of the rat jejunum. Biochim. Biophys. Acta 1280, 19–26.

Khanvilkar, K., Donovan, M.D., Flanagan, D.R., 2001. Drug transfer through mucus. Adv. Drug Deliv. Rev. 48, 173–193.

Legen, I., Kristl, A., 2001. Comparative permeability of some acyclovir derivatives through native mucus and crude mucin dispersions. Drug Dev. Ind. Pharm. 27, 669–674.

Mandel, L.J., Bacallao, R., Zampighi, G., 1993. Uncoupling of the molecular fence and paracellular gate functions in epithelial tight junctions. Nature 361, 552–555.

Poelma, F.G.J., Breäs, R., Tukker, J.J., 1990. Intestinal absorption of drugs. IV. The influence of taurocholate and L-cysteine on the barrier function of mucus. Int. J. Pharm. 64, 161–169.

Rechkemmer, G., Wahl, M., Kuschinsky, W., von Engelhardt, W., 1986. pH-microclimate at the luminal surface of the intestinal mucosa of guinea pig and rat. Pflügers Arch. 407, 33–40.

Said, H.M., Smith, R., Redha, R., 1987. Studies on the intestinal surface acid microclimate: developmental aspects. Pediatr. Res. 22, 497–499.

Shimada, T., 1987. Factors affecting the microclimate pH in rat jejunum. J. Physiol. 392, 113–127.

Takagi, M., Taki, Y., Sakane, T., Nadai, T., Sezaki, H., Oku, N., Yamashita, S., 1998. A new interpretation of salicylic acid transport across the lipid bilayer: implications of pH-dependent but not carrier-mediated absorption from the gastrointestinal tract. J. Pharmacol. Exp. Ther. 285, 1175–1180.

Ungell, A.L., Nylander, S., Sjöberg, Å., Lennernäs, H., 1998. Membrane transport of drugs in different regions of the intestinal tract of the rat. J. Pharm. Sci. 87, 360–366.

Wakabayashi, S., Shigekawa, M., Pouyssegur, J., 1997. Molecular physiology of vertebrate Na^+/H^+ exchangers. Physiol. Rev. 77, 51–73.

Wikman Larhed, A., Artursson, P., Björk, E., 1998. The influence of intestinal mucus components on the diffusion of drugs. Pharm. Res. 15, 66–71.