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European Journal of Pharmaceutics and Biopharmaceutics 56 (2003) 87–94
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

pH and energy dependent transport of ketoprofen across rat jejunum in vitro

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Received 21 August 2002; accepted in revised form 26 February 2003

Abstract

The aim of this study was to elucidate transport mechanisms of ketoprofen (monocarboxylic acid with pK_a 4.6) across rat jejunum in vitro using side-by-side diffusion cells. When the tissue was incubated on the mucosal and serosal sides with buffer of pH 7.51 (pH of the mucosal surface was 7.08), ketoprofen permeated faster in the mucosal-to-serosal than in the opposite direction. No asymmetry in transport was observed when 2 mM mucus disrupting agent 1,4-dithio-DL-threitol (pH of the mucosal surface increased to 7.21) was added to the mucosal side. Mucosal-to-serosal permeability of ketoprofen increased three times when the pH of the incubation medium was changed from 8.06 (pH of the mucosal surface was 7.34) to 6.07 (pH of the mucosal surface was 5.95), while no pH dependence was found under ATP-depletion caused by sodium azide. In the ketoprofen concentration range from 0.125 to 5 mM no saturation of transport was observed. Moreover, ketoprofen transport was not changed in the presence of 2 mM benzoate, 10 and 20 mM acetate, 20 mM L-lactate (substrates for monocarboxylate transporter 1, MCT1) and 1 mM α-cyano-4-hydroxy-cinnamic acid (an inhibitor of MCT1). These results indicate that ketoprofen is transported across rat jejunum in vitro by pH and energy dependent transport mechanisms, and most probably not by MCT1.

Keywords: Non-steroidal anti-inflammatory drugs; Microclimate pH; Monocarboxylate transporter; pH partition theory; Drug absorption

1. Introduction

Ketoprofen, a 2-arylpropionic acid derivative, is a non-steroidal anti-inflammatory drug (NSAID) which has potent inhibitory effects on prostaglandin synthesis. Ketoprofen is rapidly absorbed from the gastrointestinal tract and reaches high absolute bioavailability (>92%) [1]. Although its pharmacokinetic characteristics have been well studied, its mechanism of transport across the intestinal epithelial cells has not yet been elucidated. Some authors [2,3] have classified ketoprofen into a group of compounds which are transported across the intestinal cells by trans-cellular passive diffusion. Ketoprofen is a monocarboxylic acid with pK_a of 4.6. Absorption of weak organic acids from the intestinal tract could be explained by passive trans-cellular diffusion of the unionised form across a lipid membrane according to a pH partition theory [4,5]. This theory predicts that the permeability increases with decreasing pH, owing to an increase in the amount of the unionised form of the weak acid. It is difficult to explain the extensive absorption of ketoprofen by this mechanism because the compound is almost completely ionised in the intestinal tract, taking into account the microclimate pH at the mucosal surface of the intestine (pH 6.0–7.1) [6,7]. Although the ionised form of the weak acid can be transported by paracellular transport across tight junctions [8], this is probably not the main route of absorption for weak acids, because the area of tight junctions amounts only to about 0.01% of the whole surface.

Recently, it has been demonstrated that several monocarboxylic acids such as benzoic acid, lactic acid, atorvastatin, pravastatin and carindacillin are transported across Caco-2 cells and in rabbit jejunal brush border-membrane vesicles by a carrier mediated mechanism, termed monocarboxylate transporter 1 (MCT1), which is driven by a proton gradient across the membrane [9–12]. On the other hand, Takagi et al. [13] showed that the transport of monocarboxylic salicylic acid across the lipid bilayer of artificial liposomes was also driven by a proton gradient across the membrane and suggested that this mechanism contributes to the intestinal absorption of drugs having a monocarboxyl

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doi:10.1016/S0939-6411(03)00039-0

moiety. These two proton gradient dependent mechanisms could explain the rapid absorption of ketoprofen from the intestinal lumen.

The purpose of the present study was to elucidate the mechanism of ketoprofen absorption from the gastrointestinal tract. For this purpose, we studied the transport of ketoprofen across excised rat jejunal segments together with measurements of the microclimate pH on the mucosal surface under the conditions used in the transport experiments.

2. Materials and methods

2.1. Chemicals

Ketoprofen and α-cyano-4-hydroxy-cinnamic acid (4-CHC) were from Sigma Aldrich Chemie (Deisenhofen, Germany). Fluorescein sodium, 1,4-dithio-DL-threitol (DTT), and sodium L-lactate were purchased from Fluka (Deisenhofen, Germany). Sodium azide was obtained from Riedel-de Haën AG Seelze, (Hannover, Germany). Sodium benzoate and sodium acetate were purchased from Kemika (Zagreb, Croatia). All chemicals used in this study were of analytical grade.

2.2. In vitro intestinal transport studies

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).

The experiments were performed in a manner similar to that described previously [14]. Jejunum was excised from male Wistar rats (250–320 g) which had free access to a standard laboratory chow and tap water until 18 h before the experiment. After decapitation the small intestine was immediately excised and, prior to tissue preparation, placed into an ice-cold bubbled (carbogen, 95:5 O_2/CO_2) 10 mM solution of D-glucose in standard Ringer buffer pH 7.51 containing (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-}, for not longer than 30 min. For the experiments the jejunum located 25–60 cm distally from the pyloric sphincter was used. The tissue was rinsed with ice-cold standard Ringer buffer to remove luminal contents and cut into 3 cm long segments. Care was taken to avoid visible Peyer's patches. The intestinal segments were opened along the mesenteric border, mounted onto a special insert and placed between two EasyMount side-by-side diffusion chambers with an exposed tissue area of 1 cm^2 (Physiologic Instruments, San Diego, USA).

During the experiment the tissue was incubated on both sides with the appropriate Ringer buffer (standard Ringer buffer of pH 7.51 or modified Ringer buffers of pH 8.06, 6.47 or 6.07) containing 10 mM D-glucose at the serosal and 10 mM D-mannitol at the mucosal side. Different pH values of the Ringer buffer were achieved by changing the amount of HCO_3^-, $H_2PO_4^-$, HPO_4^{2-}. The buffers were continuously gassed with the appropriate carbogen mixture (95:5 O_2/CO_2 for buffers of pH 7.51 and 6.47 and 98:2 O_2/CO_2 for buffers of pH 8.06 and 6.07) at 37°C. The pH of the buffers was stable during the experiments. Their osmolarity was 307 mosM/kg.

After 25 min of equilibration the substance under investigation (ketoprofen or sodium benzoate) 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 final concentration of the investigated substances in the donor compartment was 250 μM, unless otherwise specified.

In some experiments, e.g. when the influence of different compounds on ketoprofen transport was studied, metabolic inhibitor sodium azide (NaN_3) (10 mM) was added to the mucosal and serosal sides, while MCT1 substrates (2 mM sodium benzoate, 10 and 20 mM sodium acetate, 20 mM sodium L-lactate), MCT1 inhibitor 4-CHC (1 mM) and the mucolytic agent DTT (2 mM) were added to the mucosal side only. The osmotic effects of these substances were balanced by addition of NaCl to the serosal side.

Samples of 250 μl were withdrawn from the acceptor compartment at 25 min intervals up to 175 or 125 min and replaced by fresh Ringer buffer containing 10 mM D-glucose (m-to-s transport) at the serosal or 10 mM D-mannitol (s-to-m transport) at the mucosal side.

The chambers were equipped with two pairs of Ag/AgCl-electrodes connected to the chambers via 3 M KCl/3.5% agar bridges; one pair of electrodes was used for measuring trans-epithelial potential difference (PD), and one pair for passing current. The tissues were short-circuited to zero PD by a multichannel voltage–current clamp (model VCC MC6, Physiologic Instruments, USA). The tissue viability and integrity were checked by monitoring PD, short circuit current (I_{sc}) and trans-epithelial electrical resistance (TEER) 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. In viable tissues, PD, I_{sc} and TEER did not change significantly over time. The background potential (asymmetry of the electrodes and liquid junction potential) was compensated before mounting the tissue in the diffusion chamber system. Additionally, I_{sc} and TEER were corrected for fluid resistance. TEER was determined according to Ohm's law.

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 digital pH

meter (model MA-5736, Iskra, Ljubljana, Slovenia) equipped with a flat membrane pH microelectrode (MI-406, tip diameter 1.5 mm, Microelectrodes, Inc., Bedford, USA) and a reference microelectrode (MI-402, Microelectrodes, Inc.). The tissue was first incubated for at least 1 h in the diffusion chambers under the same conditions as used in the transport experiments. The insert with the mounted tissue was then placed into a thermostated bath (37°C) with the mucosal surface upwards and perfused with the appropriate Ringer buffer, which was gassed with carbogen and kept at 37°C. After determining the pH 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 observed by a change in pH. The electrode was then lowered by an additional 0.5 mm. Stable pH readings were achieved within 3–4 min.

The procedure of pH determination did not affect the tissue viability, which was checked by monitoring the electrical parameters of the tissue before and after pH measurements. The pH of the mucosal surface did not significantly change over time (up to 2.5 h) during incubation in the diffusion chambers.

2.4. Analytical procedure

The concentrations of ketoprofen, benzoate and fluorescein in the samples from the transport experiments were analysed by HPLC (Series 1100, Hewlett Packard, Waldbron, Germany). A Eurospher C-8 column (5 μm, 250 × 4 mm) (Bia Separations, Ljubljana, Slovenia) was used at 35°C. The mobile phase for ketoprofen and fluorescein consisted of 15% acetonitrile and 85% phosphate buffer (pH 7.5). The mobile phase for benzoate consisted of 33% acetonitrile and 67% 10 mM H_3PO_4 (pH was adjusted with triethylamine to 2.75). Ketoprofen and benzoate were detected at 262 and 230 nm, respectively, using a diode array detector. Fluorescein was detected by fluorescence (λ_{EX} 487 nm, λ_{EM} 510 nm) (model RF-535, Shimadzu, Kyoto, Japan).

2.5. Data analysis and statistics

Apparent permeability coefficients (P_{app}) were calculated according to the equation:

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

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

Results are expressed as means ± SEM. Two-group comparisons were analysed by unpaired two-tailed Student's t-test. ANOVA (single factor) was used to evaluate the effect of ketoprofen concentration on the P_{app} values of fluorescein.

3. Results and discussion

3.1. Microclimate pH on the mucosal surface of the rat jejunum in vitro

The transport of weak electrolytes across intestinal tissue depends on the microclimate pH at the mucosal surface in situ [5] and in vitro [15]. In order to investigate its role in the transport of ketoprofen across rat jejunum in vitro we measured the pH of the intestinal surface under the same conditions as those used in the transport experiments. The results (Table 1) demonstrate the presence of an acidic microclimate layer in the vicinity of the mucosal surface of the rat jejunum in vitro. The pH of the microclimate is significantly lower than that of the luminal buffer. The highest ($\Delta pH = 0.72$) and the smallest ($\Delta pH = 0.12$) differences between the luminal and the microclimate pH values were obtained when luminal pH was 8.06 and 6.07, respectively. In our studies the microclimate pH changed by 1.39 pH units (from 7.34 to 5.95) when the pH of the luminal buffer decreased by 1.99 pH units (from 8.06 to 6.07). A similar influence of the luminal buffer pH on the microclimate pH was observed in the rat jejunum in vivo by Lucas [16] and in the guinea-pig colon in vitro by Genz et al. [17]. On the other hand, Högerle and Winne [5] and Elbert et al. [15] observed that the microclimate pH of the rat jejunum in situ and in vitro was only moderately influenced by changes of pH in the luminal buffer. These different observations could be ascribed to different experimental procedures (e.g. time of tissue incubation and type of luminal buffers).

The addition of the respiratory chain inhibitor sodium azide to the bathing solution significantly increased the pH of the mucosal surface (Table 1). This agent binds tightly to

Table 1
The effects of the pH of the incubation medium and of sodium azide (NaN_3) on the surface pH of the rat jejunal mucosae in vitro[a]

pH of incubation medium	pH of mucosal surface	pH of mucosal surface after NaN_3 addition[b]
8.06 ± 0.02	7.34 ± 0.02[c]	7.61 ± 0.07[c,d]
7.51 ± 0.01	7.08 ± 0.02[c]	7.35 ± 0.01[c,d]
6.47 ± 0.01	6.02 ± 0.03[c]	6.35 ± 0.02[c,d]
6.07 ± 0.02	5.95 ± 0.04[c]	6.08 ± 0.03[d]

[a] Data are expressed as means ± SEM of at least six determinations.
[b] Sodium azide (10 mM) was present in the incubation medium.
[c] pH is significantly different from the pH of incubation medium ($P < 0.05$).
[d] pH values are significantly different from those in the absence of NaN_3 in the incubation medium (middle column) ($P < 0.05$).

the cytochrome oxidase complex, thereby blocking all electron transport [18]. It has been shown that 10 mM sodium azide markedly decreases intracellular ATP concentration [19]. One can thus conclude that there is an energy dependent mechanism for controlling mucosal surface pH. Similar effects of other metabolic inhibitors (dinitrophenol and iodoacetate) on the microclimate pH were observed by Said et al. [7].

Treatment of the mucosal side of the tissue with the disulphide reducing agent DTT (2 mM) at luminal pH 7.51 significantly increased the pH of the mucosal surface from 7.08 ± 0.02 ($n = 6$) to 7.21 ± 0.02 ($n = 6$, $P < 0.001$). Reduction of the disulphide bonds between glycoprotein sub-units in the mucus structure is known to affect microclimate pH [20].

3.2. Transport studies

Polarised transport of ketoprofen across rat jejunum in vitro was observed when both sides of the tissue were bathed with standard Ringer buffer of pH 7.51 (Fig. 1, control). The m-to-s permeability [$P_{app} = 1.91 \times 10^{-5} \pm 0.14 \times 10^{-5}$ cm/s ($n = 3$)] was significantly higher ($P < 0.05$) than s-to-m permeability [$P_{app} = 1.14 \times 10^{-5} \pm 0.14 \times 10^{-5}$ cm/s ($n = 3$)], while no polarisation was found when the mucosal side was treated with DTT [$P_{app} = 1.47 \times 10^{-5} \pm 0.41 \times 10^{-5}$ cm/s ($n = 3$) and $P_{app} = 1.44 \times 10^{-5} \pm 0.26 \times 10^{-5}$ cm/s ($n = 3$) for m-to-s and s-to-m transport, respectively] (Fig. 1). This indicates that an intact mucus layer and consequently lower microclimate pH is very important for the observed polarised ketoprofen transport.

Takagi et al. [13] pointed out the role of an inwardly directed proton gradient across the lipid membrane in the absorption of drugs having a monocarboxylic moiety. They showed that such a gradient (pH extraliposomal < pH intraliposomal) across an artificial liposome membrane resulted in the rapid uptake of the monocarboxylic salicylic acid. When both sides of the liposomal membrane were adjusted to the same pH, the uptake of salicylic acid decreased, because intraliposomal salicylic acid initially taken up as an unionised form quickly reaches equilibrium with the extraliposomal salicylic acid [13]. In our case, under the control conditions, an inwardly directed proton gradient existed across the apical membrane of the enterocytes, because the pH of the mucosal surface (7.08, Table 1) was lower than the intracellular pH, which was about 7.2 [21,22]. Consequently, a more pronounced gradient of the unionised ketoprofen across the brush border membrane in the m-to-s direction could be maintained, which facilitates the trans-cellular transport of ketoprofen in this direction. At the same time, the inwardly directed proton gradient across the apical membrane hinders transport in the s-to-m direction, because it reduces the gradient of the non-ionised ketoprofen in this direction. Treatment of the mucosal side of the epithelium with DTT diminished the proton gradient across the apical membrane (pH of the mucosal surface increased to 7.21) and consequently diminished polarisation of ketoprofen transport across the intestinal tissue.

The influence of the mucosal surface pH on the m-to-s transport of ketoprofen was further examined. We performed transport experiments in incubation media at different pH values (Fig. 2, control). Considering the effect of the luminal buffer pH on the mucosal surface pH (Table 1), it can be concluded that the transport of ketoprofen increased with decreasing pH of the mucosal surface. This further indicates that the inwardly directed proton gradient across the brush border membrane can be considered as a driving force for ketoprofen trans-cellular transport. In the investigated pH interval (6.07–8.06), I_{sc} and TEER values of the tissue were in the expected range (Table 2). In addition, the increase of I_{sc} values after the addition of D-glucose to the mucosal side of the epithelium demonstrates that the active transport of D-glucose (co-transport with sodium) was not affected (Table 2). These results show that the intestinal tissue was not damaged at the lower pH of the incubation medium during the experiments.

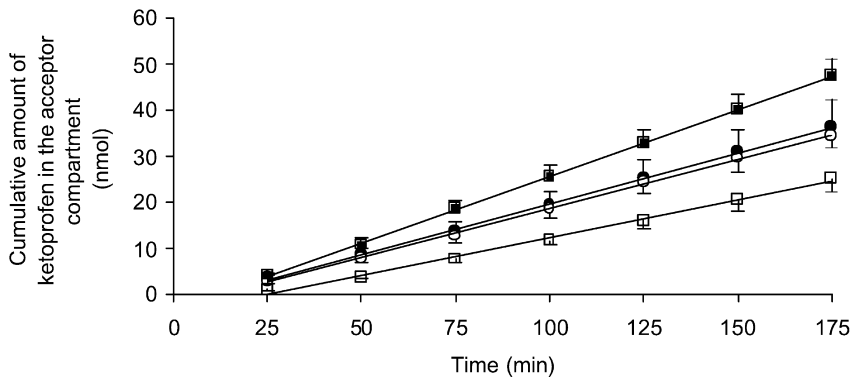

Fig. 1. Amount of ketoprofen ($C_0 = 250$ μM) transported across rat jejunum in vitro as a function of time. The pH of the mucosal and serosal bathing solution was 7.51. Transport was examined in the mucosal-to-serosal (■,●) and in the opposite direction (□,○), without (control) (■,□) and with (●,○) 2 mM of DTT in the mucosal compartment. Data are presented as means ± SEM of three experiments.

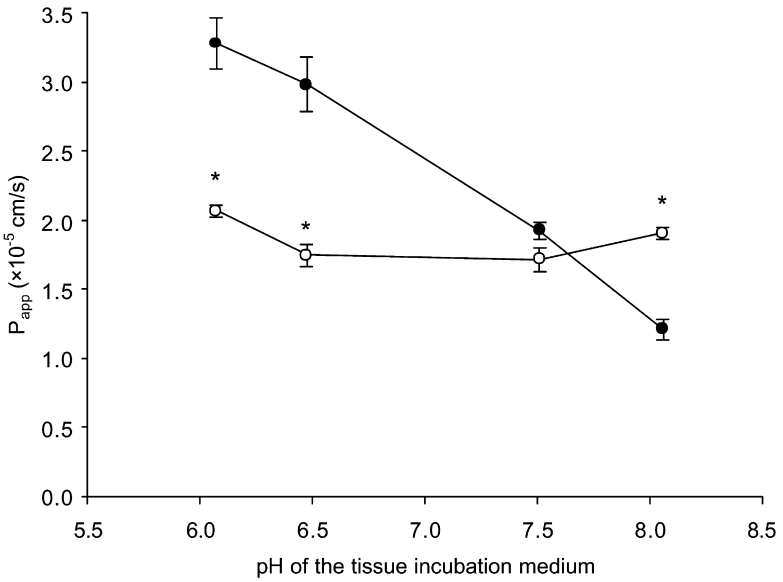

Fig. 2. Mucosal-to-serosal transport of ketoprofen ($C_0 = 250$ μM) across rat jejunum in vitro as a function of pH of the incubation media (pH in the mucosal compartment was the same as in the serosal compartment). The pH dependence of ketoprofen transport was studied without (●) (control) and with (○) 10 mM of sodium azide in the mucosal and serosal compartment. Data are presented as means ± SEM of at least three experiments. *Significantly different from the control value ($P < 0.0001$).

In contrast, no influence of the incubation medium pH on ketoprofen transport was observed when the metabolic inhibitor sodium azide was present in the bathing solutions (Fig. 2), although the mucosal surface pH was changed (Table 1). In this case, the transport of ketoprofen was probably affected by changes in the intracellular pH of enterocytes. In jejunal enterocytes the intracellular pH is predominantly regulated by Na^+/H^+ exchanger [21]. It has been demonstrated that cellular ATP-depletion caused by metabolic inhibitors reduces the exchange activity of Na^+/H^+ exchanger dramatically in several cell types [22,23], due to a modulation of an intracellular proton-dependent regulatory mechanism [23]. Additionally, depletion of ATP prevents the activity of Na^+-K^+-ATPase, which also affects Na^+/H^+ exchange activity since the Na^+/H^+ exchanger is a secondary active transporter driven by the inwardly directed sodium gradient across the cell membrane generated by Na^+-K^+-ATPase [22]. Therefore, in the ATP-depleted cells the intracellular pH cannot be regulated, which leads to cellular acidification. Consequently, no inwardly directed proton gradient across the brush border membrane can be expected at any pH, leading to the decreased (the only exception is the P_{app} value at pH 8.06) and pH independent transport of ketoprofen (Fig. 2).

Table 2
The effect of pH of the incubation medium on the electrical parameters of rat jejunum in vitro[a]

Incubation medium pH	TEER (Ω cm^2)	I_{sc} (μA/cm^2)	I_{sc}^{GLU} (μA/cm^2)
8.06	33.3 ± 2.2	39.3 ± 2.7	79.5 ± 14.1[b]
7.51	23.8 ± 1.7	40.8 ± 6.0	128.9 ± 9.5[b]
6.47	24.9 ± 1.0	32.8 ± 2.3	97.2 ± 8.1[b]
6.07	30.8 ± 2.3	34.4 ± 5.1	71.3 ± 14.9[b]

TEER-trans-epithelial electrical resistance, I_{sc}-short circuit current, I_{sc}^{GLU}-short circuit current after the addition of D-glucose (25 mM) to the mucosal side of the epithelium. TEER and I_{sc} from one experiment are the average values of determinations obtained at 25 min intervals. I_{sc}^{GLU} was measured 5 min after the addition of D-glucose at the end of experiment.

[a] Data are presented as means ± SEM from at least five experiments.

[b] The increase of I_{sc} is significant ($P < 0.05$).

The effect of sodium azide on tissue viability was seen as abolishing PD and I_{sc} in approximately 10 min after the addition of sodium azide to the bathing solution and preventing an increase in I_{sc} after the addition of D-glucose to the mucosal side. Furthermore, TEER values decreased rapidly during the experiments with sodium azide, which is in agreement with the observation that ATP-depletion affects tight junction control and consequently increased paracellular permeability [24]. Accordingly, we have observed that the P_{app} values of the paracellular transport marker fluorescein for m-to-s transport increased significantly ($P < 0.05$) from $5.03 \times 10^{-6} \pm 0.25 \times 10^{-6}$ cm/s ($n = 18$) to $14.4 \times 10^{-6} \pm 1.45 \times 10^{-6}$ cm/s ($n = 3$) at pH 7.51 as a result of sodium azide treatment. This increased paracellular permeability of the intestine in the presence of sodium azide suggests that ketoprofen transport across the intestine under ATP-depleted conditions is predominantly paracellular, and that this paracellular transport is independent of pH of the incubation medium (Fig. 2). The increased paracellular permeability of the intestine in the presence of sodium azide could also explain the higher P_{app} value of ketoprofen obtained at

pH 8.06 under ATP-depleted conditions than in the control (Fig. 2). Thus, at luminal pH 8.06, predominantly paracellular transport of ketoprofen is expected, owing to the lack of an inwardly directed proton gradient across the brush border membrane, since the pH of the mucosal surface (7.34, Table 1) is higher than the intracellular pH (7.2).

On the other hand, it has been demonstrated that under the inwardly directed proton gradient across the brush border membrane several monocarboxylic acids could be transported across the intestinal epithelia (rat, rabbit, human) by a proton/monocarboxylate co-transporter 1 (MCT1) with K_m values ranging in general between 1–5 mM [9–12,25]. MCT1 has already been identified by immunohistochemical analysis on the apical membrane of the rat jejunal enterocytes [25]. Therefore, it is possible that this transporter might contribute to the overall transport of ketoprofen across the rat jejunum in vitro. To evaluate the possible contribution of a carrier mediated mechanism to the transport of ketoprofen the effect of ketoprofen concentration on the m-to-s transport was studied. Fig. 3 shows that the transport of ketoprofen at pH 7.51 was linear in the concentration range tested (0.125–5 mM), suggesting that ketoprofen is transported by a non-saturable process. Care must be taken when studying the concentration dependence of NSAIDs transport, because NSAIDs increase the intestinal permeability in a concentration dependent manner due to the opening of tight junctions [26–28]. This might mask the eventual saturation of ketoprofen transport at higher concentrations and consequently underestimate the contribution of a carrier mediated process in ketoprofen transport. However, the influence of ketoprofen in the concentration range between 0.125 and 5 mM on the P_{app} values of a hydrophilic transport marker fluorescein was not significant (ANOVA, $P > 0.05$). The average P_{app} value of fluorescein in this ketoprofen concentration range was $6.27 \times 10^{-6} \pm 0.37 \times 10^{-6}$ cm/s ($n = 25$). Higher concentrations of ketoprofen (7.5 and 10 mM) markedly increase the P_{app} values for fluorescein [28]. The concentration dependence of ketoprofen transport was not studied at a lower pH of the incubation medium because of the solubility limit of ketoprofen (less than 3 mM at pH 6.47) and because of the pronounced cytotoxicity of ketoprofen at lower pH. Ketoprofen 1.5 mM at pH 6.47 already affects tissue viability, shown by low PD values, between 0 and −0.3 mV, and lack of I_{sc} increase after the addition of D-glucose to the mucosal side of the tissue at the end of the experiment.

To further characterise the ketoprofen transport mechanism in the m-to-s direction the effect of different monocarboxylates on ketoprofen transport was studied (Table 3). Benzoate, acetate and L-lactate are MCT1 substrates [25,29], while 4-CHC is an MCT1 inhibitor, which binds strongly to the transporter without being translocated [29]. First, we evaluated the transport of benzoate. The addition of 20 mM acetate significantly decreased benzoate transport (Table 3). On the other hand, the addition of 2 mM benzoate, 10 and 20 mM acetate, 20 mM L-lactate or 1 mM 4-CHC did not significantly influence the rate of ketoprofen transport. These results suggest that ketoprofen and the MCT1 substrate benzoate are transported across rat jejunum by different mechanisms.

The concentrations of MCT1 substrates used to competitively inhibit MCT1 transport are usually higher than 10 mM [10,11]. In our experiments benzoate could not be used at such a high concentration, because it affected the tissue viability, seen as low PD (between 0 and −0.3 mV) and lack of I_{sc} increase after the addition of D-glucose to the mucosal side of the tissue. The concentration of MCT1 inhibitor 4-CHC was 1 mM, because it has been demonstrated that this concentration is high enough to inhibit MCT1 mediated transport of 10 mM L-lactate in *Xenopus laevis* oocytes expressing MCT1 [29] and 0.5 mM butyrate in Caco-2 cells [30].

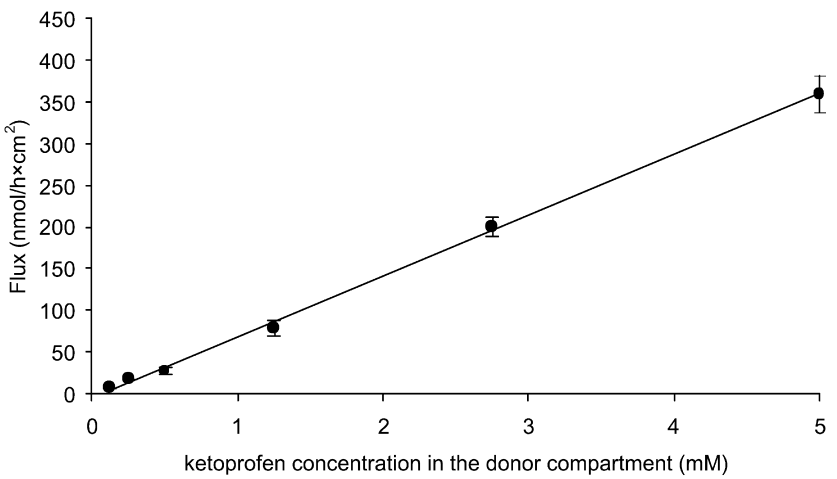

Fig. 3. Concentration dependence of ketoprofen mucosal-to-serosal transport across rat jejunum in vitro. pH of the mucosal and serosal bathing solution was 7.51. Each point represents the mean ± SEM of at least three experiments.

Table 3
The effect of different monocarboxylates on the m-to-s transport of benzoate ($C_0 = 250$ μM) and ketoprofen ($C_0 = 250$ μM) across rat jejunum in vitro[a]

Investigated substance	Monocarboxylate	Concentration of monocarboxylate (mM)	Apparent permeability[b], P_{app} ($\times 10^{-5}$ cm/s)
Benzoate	Control[c]		5.65 ± 0.54
	Acetate	20	3.38 ± 0.17[d]
Ketoprofen	Control[c]		2.93 ± 0.20
	Benzoate	2	2.47 ± 0.48
	Acetate	10	2.65 ± 0.07
	Acetate	20	2.64 ± 0.44
	L-Lactate	20	3.23 ± 0.10
	4-CHC	1	2.96 ± 0.53

[a] Monocarboxylates were added to the mucosal compartment. The pH of the incubation medium on both sides of the tissue was 6.47.
[b] Data are presented as means ± SEM of at least three experiments.
[c] Without monocarboxylates in the incubation medium. Only the investigated substance is examined.
[d] Significantly different from the corresponding control ($P < 0.01$).

In summary, we have demonstrated that ketoprofen transport across rat jejunum in vitro is influenced by the pH of the mucosal surface and by ATP-depletion. The results suggest that the inwardly directed proton gradient across the brush border membrane, maintained by an energy dependent mechanism, can be considered as the driving force for ketoprofen trans-cellular transport across intestinal epithelia. This pH dependent non-MCT1 mediated transport mechanism, which was proposed for monocarboxylic acid type drugs by Takagi et al. [13], might explain the rapid absorption of ketoprofen from the intestinal tract. However, further studies are needed to investigate the role of other possible transporters for ketoprofen absorption, such as other members of the MCT family, MCT5 and MCT8, which were identified in human small intestine by Northern-blot analysis [31,32] but have not yet been functionally characterised.

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