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Ketoprofen-induced intestinal permeability changes studied in side-by-side diffusion cells

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Abstract

It is known that non-steroidal anti-inflammatory drugs (NSAIDs) increase intestinal permeability. Increased intestinal permeability is believed to result from the opening of tight junctions because of NSAID-induced reduction of prostaglandin synthesis and/or energy-depletion. In this study, ketoprofen-induced changes in intestinal permeability were evaluated by measuring tissue electrical parameters, namely tissue electrical resistance (TER), short circuit current (I_{sc}) and transepithelial potential difference (PD), and the transport of a paracellular marker, fluorescein, across rat jejunum in-vitro. Ketoprofen, added to the mucosal side of the tissue, decreased TER and increased fluorescein transport in a concentration-dependent manner. I_{sc} values and the active transport of D-glucose were not affected at ketoprofen concentrations of less than 5 mM. Higher ketoprofen concentrations decreased I_{sc} values and diminished active transport of D-glucose, while transport of fluorescein increased markedly. Similar effects on intestinal properties were observed when the metabolic inhibitor sodium azide was added to the incubation medium. The results of this study suggest that the increased intestinal permeability observed at lower ketoprofen concentrations (< 5 mM) is most probably a consequence of reduced prostaglandin tight junction control, whereas at higher concentrations, ATP depletion caused by ketoprofen seems to be the major mechanism for increased intestinal permeability.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used drugs, with proven efficacy as analgesics, antipyretic and anti-inflammatory agents. However, there is concern about their side-effects, the most frequent being gastrointestinal disturbances (Hawkey 1988). Numerous studies on gastroduodenal damage caused by NSAIDs have been reported and there is also strong evidence that NSAIDs frequently cause small intestinal inflammation associated with blood and protein loss (Bjarnason et al 1993). In addition, NSAIDs may occasionally cause small intestinal perforation, ulcers and strictures, requiring surgery (Bjarnason et al 1993).

The NSAID-induced increased small intestinal permeability measured by poorly absorbed hydrophilic molecules in humans and rats appears to be a triggering factor in the development of inflammation, facilitating the invasion of bacteria or the action of other aggressive agents such as bile acid or certain food components (Bjarnason et al 1986; Ford et al 1995; Davies et al 1994, 1996; den Hond et al 1999). Indeed, it was shown that NSAIDs-induced increased [^{51}Cr]EDTA permeability across rat intestine was associated with increased intestinal ulceration (Ford et al 1995).

Although there is much evidence that NSAIDs increase intestinal permeability in humans and animal models, the mechanism by which NSAIDs increase intestinal permeability is still not clear. The purpose of the present study was to gain an insight into the mechanism by which the potent NSAID, ketoprofen, increases intestinal permeability. For this purpose, we measured intestinal electrical parameters and the transport of a hydrophilic model compound, fluorescein, across excised rat jejunal segments in side-by-side diffusion cells.

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Materials and Methods

Chemicals

Ketoprofen was purchased from Sigma Aldrich Chemie (Deisenhofen, Germany). Fluorescein sodium was obtained 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.

In-vitro intestinal transport studies

Guidelines and legislative regulations on the use of animals for scientific purposes were followed.

Rat jejunum was obtained from male Wistar rats (250–320 g) that had been fasted for 24 h before the experiments. After decapitation, the small intestine was immediately excised and placed into ice-cold bubbled (carbogen; O_2/CO_2, 95:5) Ringer buffer (composition in mM: 140.6 Na^+, 5 K^+, 1.2 Ca^{2+}, 1.2 Mg^{2+}, 121.8 Cl^-, 25 HCO_3^-, 0.4 $H_2PO_4^-$ and 1.6 HPO_4^{2-}) for not longer than 40 min. For the experiments, jejunum 25–60 cm distal from the pyloric sphincter was used. The intestine was rinsed with ice-cold standard Ringer buffer to remove luminal contents and cut into 3-cm long segments, excluding visible Peyers 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 an exposed tissue area of 1 cm^2 (Physiologic Instruments, San Diego, CA).

Both sides of the tissue were bathed with Ringer buffer supplemented with 10 mM glucose at the serosal side and 10 mM mannitol at the mucosal side. The incubation medium was gassed with carbogen and kept at 37°C; the pH of the incubation medium during the experiment was 7.51.

After 25 min equilibration time, the solution on the mucosal side was replaced with fresh Ringer buffer containing 5 μM fluorescein, 10 mM mannitol and the appropriate concentration of ketoprofen. The final volume of the solution in each compartment was 2.5 mL.

Samples of 250 μL were withdrawn from the serosal compartment at 25-min intervals up to 175 min and replaced with fresh Ringer buffer containing 10 mM glucose.

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 transepithelial potential difference (PD), and the other pair for passing current. During the experiments the tissues were short-circuited to zero PD by a multichannel voltage-current clamp (model VCC MC6; Physiologic Instruments). Tissue viability and integrity were checked by monitoring PD, short circuit current (I_{sc}) and tissue electrical resistance (TER) every 25 min during the experiment and additionally by recording the increase of I_{sc} and PD after the addition of D-glucose (final concn 25 mM) to the mucosal compartment at the end of experiments (I_{sc}^{glu} and Pd^{glu}). I_{sc} and TER were corrected for fluid resistance before mounting the tissue in the diffusion chamber system.

Analytical procedure

The concentration of fluorescein in the samples from the transport experiments was analysed using a HPLC system (Series 1100; Hewlett Packard, Waldbron, Germany). The column Eurospher C-8 (5 μm, 250 × 4 mm) (Bia Separations, Ljubljana, Slovenia) was applied at 35°C. The mobile phase consisted of 15% acetonitrile and 85% phosphate buffer (pH 7.5). Fluorescence was detected at excitation and emission wavelengths of 487 nm and 510 nm, respectively, using a fluorescence detector (model RF-535, Shimadzu, Kyoto, Japan).

Data analysis

The apparent permeability coefficient (P_{app}) of fluorescein was calculated according to the equation:

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

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

All data are presented as means ± s.d. Unless otherwise indicated, statistical significance was taken at the $P < 0.05$ level. Kruskal–Wallis tests were used to evaluate the influence of ketoprofen and sodium azide on P_{app}, TER, I_{sc}, I_{sc}^{glu}, PD and PD^{glu}. Mann–Whitney *U*-tests with Holm's sequential Bonferroni method for control of type 1 error were conducted for pairwise comparisons. Wilcoxon signed rank tests were applied to assess the effect of the addition of glucose on I_{sc} and PD.

Results and Discussion

The influence of ketoprofen on rat intestinal tissue was evaluated by fluorescein transport. This hydrophilic compound is suggested to be transported across intestinal epithelia mainly through the paracellular route (Bock et al 1998; Gaillard & de Boer 2000), but only at neutral or alkaline pH of the transport medium (Kuwayama et al 2002). Ketoprofen-induced changes in the intestinal permeability were additionally evaluated by measuring the tissue electrical parameters, which reflected tissue integrity and viability: TER could be related to the state of the epithelial tight junctions; I_{sc} is a measure for active transport of ions from one side to another and depends mainly on the Na^+/K^+-ATPase activity; and PD is a product of I_{sc} and TER. In addition, the increase of PD and I_{sc} after the addition of D-glucose to the mucosal side of the epithelium reflects the ability of the epithelium to actively transport D-glucose. D-Glucose is transported across the apical surface of the enterocytes by a two-Na^+/one-D-glucose symporter, secondary active transporter, which is driven by the sodium gradient across cell membrane generated by Na^+/K^+-ATPase (Lodish et al 2000).

Ketoprofen in the bathing solution at the mucosal side of the tissue reduced TER values (Table 1) and increased the transport of fluorescein (Figure 1). The most pronounced effect on fluorescein transport and TER was

Table 1 Effect of ketoprofen concentration and sodium azide on the electrical parameters of the rat jejunum in-vitro.

Conditions	TER (Ω cm^2)	PD (mV)	PD^{glu} (mV)	I_{sc} (μA cm^{-2})	I_{sc}^{glu} (μA cm^{-2})
Control	33.5±7.4	−1.6±0.4	−2.6±0.9†	50.8±11.3	100.3±46.1†
+0.25 mM ketoprofen	23.5±4.0*	−1.2±0.3*	−3.3±0.6†	49.6±12.5	117.7±33.5†
+1.25 mM ketoprofen	21.4±4.7*	−0.91±0.12*	−2.6±0.6†	45.2±11.2	138.8±45.8†
+2.75 mM ketoprofen	23.2±0.8	−1.0±0.12*	−2.4±0.5	46.8±4.6	133.3±30.6
+5.0 mM ketoprofen	23.5±1.6	−0.68±0.06*	−1.0±0.2*	29.0±1.8*	40.0±9.0*
+7.5 mM ketoprofen	20.2±1.4*	−0.49±0.01*	−0.43±0.12*	24.7±3.3*	27.4±6.9*
+10 mM ketoprofen	20.6±3.1*	−0.48±0.17*	−0.40±0.20*	25.0±13.2*	22.9±13.5*
+sodium azide[a]	19.2±5.2*	−0.24±0.16*	−0.23±0.06*	12.0±2.1*	11.0±2.8*

TER, PD and I_{sc} are the tissue electrical resistance, transepithelial potential difference and short circuit current, respectively, during the experiments; PD^{glu} and I_{sc}^{glu} are the transepithelial potential difference and short circuit current determined 5 min after the addition of D-glucose to the mucosal side of the epithelium (final concn 25 mM) at the end of experiments. Ketoprofen was present on the mucosal side of the tissue. Data are means±s.d. from at least three experiments. The influence of ketoprofen concentration and sodium azide on all investigated electrical parameters was significant ($P < 0.01$ for each electrical parameter). [a]Sodium azide was present in the incubation medium at 10 mM on both sides of the tissue. *$P < 0.05$, significantly different compared with the control value. †$P < 0.05$, significantly different compared with PD or I_{sc} values before the addition of D-glucose to the mucosal side.

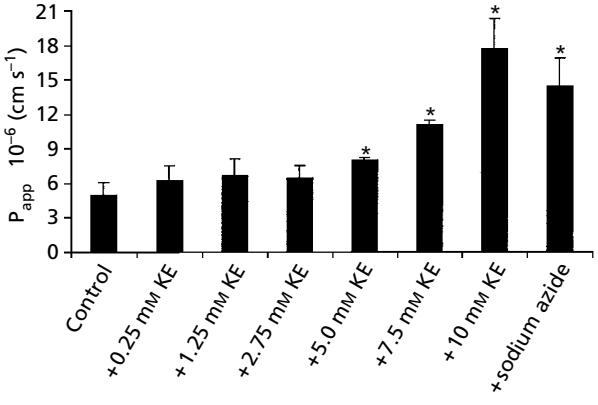

Figure 1 Effect of different ketoprofen concentrations and sodium azide on the intestinal permeability (P_{app}) of fluorescein. Ketoprofen (KE) was applied to the mucosal side, while sodium azide was present in the incubation medium at 10 mM on both sides of the tissue (for the experiment with sodium azide). Data are means±s.d. from at least three experiments. The influence of ketoprofen concentration and sodium azide on P_{app} values was significant ($P < 0.001$). *$P < 0.05$, significantly different compared with the control value (without ketoprofen or sodium azide).

observed at the highest (7.5 and 10 mM) concentrations of ketoprofen. The results presented in Table 1 and Figure 1 indicate that ketoprofen affects epithelial tight junction control and consequently increases intestinal permeability. This is in agreement with the two major mechanisms that attempt to explain NSAID-induced increased intestinal permeability. First, NSAIDs are cyclooxygenase inhibitors and may increase intestinal permeability (i.e. opening of tight junctions) by a reduction of prostaglandin synthesis (Bjarnason et al 1989). Prostaglandins play an important role in epithelial tight junctions control, probably via the second messengers Ca^{2+} and cAMP (Krugliak et al 1990; Blikslager et al 1997). Second, it was suggested that NSAIDs inhibit mitochondrial oxidative phosphorylation, and that the resultant shortage of ATP causes cell damage and loss of control of tight junctions (Bjarnason et al 1993). However, the results obtained in the present study indicate that, at lower concentrations of ketoprofen (< 5 mM), the increased intestinal permeability was most probably not the consequence of the reduced intracellular ATP level. The results in Table 1 show that over this concentration range, I_{sc} and I_{sc}^{glu} values were not significantly different from the control, indicating that the intracellular ATP level was not affected because the activity of Na^+/K^+-ATPase was not disturbed.

On the other hand, higher ketoprofen concentrations (5, 7.5 and 10 mM) in the bathing solution significantly decreased PD and I_{sc} (Table 1). Additionally, no significant increase of I_{sc} or PD after the addition of D-glucose to the mucosal side of the epithelium at the end of experiment was observed (Table 1). Similar effects on the tissue electrical parameters and fluorescein transport were observed when the intestinal rat tissue was incubated with a respiratory chain inhibitor, sodium azide (Table 1; Figure 1). One can therefore conclude that the increased intestinal permeability observed at these higher ketoprofen concentrations resulted mainly from ATP depletion.

The results in Table 1 show that TER decreased pronouncedly after ketoprofen addition to the bathing solution (from 33.5 to 23.5 Ω cm^2), and with increasing ketoprofen concentration, TER was between 23.5 and 20.2 Ω cm^2. There was no significant decrease in TER with ketoprofen concentration, while the transport of fluorescein at ketoprofen concentrations higher than 5 mM increased markedly compared with lower ketoprofen concentrations (Figure 1). This can be explained by the fact, that TER is a summation of epithelial resistance, which depends on the conditions of tight junctions, and subepithelial resistance (Hemlin et al 1988). Increasing lamina propria oedema in the subepithelial region, which was associated with the reduced viability of the human ileum in-vitro (Söderholm

et al 1998), could be the reason for the insignificant decrease of TER values during the experiments with the highest ketoprofen concentrations.

It was shown previously that NSAID-associated changes in rat intestinal permeability are similar to those described in humans (Davies et al 1994). Therefore, the results obtained in our study using rat tissue in-vitro may have significant clinical relevance. Ford et al (1995) reported strong and positive correlation between NSAID-induced intestinal permeability and intestinal ulceration in the rat. Therefore, in order to avoid severe ulceration, one should avoid high individual doses of ketoprofen, especially doses exceeding 5 mM ketoprofen, in the intestinal lumen (doses >300 mg, assuming 200–250 mL water with dose). Ketoprofen at concentrations greater than 5 mM markedly increased intestinal permeability (Figure 1) and may lead to pronounced intestinal ulceration. The usual daily adult doses (100–300 mg) should be divided into 3–4 individual doses. Extended-release dosage forms of ketoprofen might therefore be more appropriate for the treatment of chronic diseases. The prolonged exposure of certain intestinal regions to low ketoprofen concentrations, which could be the case when extended-release dosage forms are used, is probably not the limiting factor. We have observed that the appearance rate of fluorescein on the acceptor side of the tissue was linear up to 175 min (duration of the experiment; data not shown) at all ketoprofen concentrations. This indicates that the ketoprofen concentration in the intestinal lumen, and not the prolonged exposure of the intestinal segments to ketoprofen, is responsible for the increased intestinal permeability.

In conclusion, the results from the present study show that ketoprofen affects intestinal permeability in a concentration-dependent manner. The increased intestinal permeability induced by low (< 5 mM) concentrations of ketoprofen in the intestinal lumen is probably a consequence of reduced prostaglandin synthesis, whereas at higher ketoprofen concentrations (> 5 mM), ATP depletion is the major reason for the increased intestinal permeability. This may be useful information in the development of appropriate strategies for the reduction of NSAID-related gastrointestinal disturbances. Additionally, the Ussing chamber technique was shown to be a suitable tool for the evaluation of NSAID-associated intestinal permeability changes. It gives information about permeability of the intestine to hydrophilic transport markers and on tissue viability.

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