비스테로이드성 소염제의 소장 내흡수에서 monocarboxylic acid transporters의 역할

Role of Monocarboxylic Acid Transporters in the intestinal absorption of Nonsteroidal Anti-inflammatory Drugs

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김 명 길

지도교수: 한 효 경

조선대학교 대학원 약학과

이번연구는 Caco-2cell서의 비스테로이드성 소염제의 세포흡수 기전을 찾는 것이다. Caco-2 cell서 Diflunisal, diclofenac, ketoprofen and naproxen 4가지약물은 Benzoic acid에 대해 강한 억제를 나타내며 IC50 는 0.05-0.44다.

Ketoprofen and naproxen 두 약물은 Benzoic acid의세포막 투과를 억제하였으며 Ki가 각각 0.38mM과 0.22mM이다.

Ketoprofen 과 naproxen 약물은 세포 내 흡수에서 고농도일 때 포화상태에 도달했기에 농도에도 의존하는 것을 알 수 있다. 그리고 ketoprofen은 Benzoic acid와 Lactic acid에 의해 세포 내 축적이 감소된다.

그래서 MCT1가 carboxylic acid 구조를 가진 비스테로이성 소염제의 수송체 라는 것을 알 수 있다.

쥐에서 ketoprofen(1mg/kg)을 각각 benzoic acid(10mg/kg)와 lactic acid(10mg/kg)와 동시경구 투여한 것을 대조군으로 하고 ketoprofen(1mg/kg)만 경구 투여한 것을 통제집단으로 하고 비교했는데 대조군에서의 Cmax와AUC는 감소되고 $T_{1/2}$와Tmax는 영향이 크게 없었다($P<0.05$) Benzoic acid와 lactic acid의 수송체는 monocarboxylic acid transporters다. 그러므로 여기에서 benzoic acid와 lactic acid가 ketoprofen의 흡수를 저해한다는 것을 알 수 있고, ketoprofen의 수송체가 monocarboxylic acid transporters것을 알 수 있다.

Abstract

Role of Monocarboxylic Acid Transporters in the intestinal absorption of Nonsteroidal Anti-inflammatory Drugs

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The present study aims to investigate the cellular uptake mechanism of Nonsteroidal anti-inflammatory drugs (NSAIDs) in Caco-2 cells. Diflunisal, diclofenac, ketoprofen and naproxen exhibited the strong inhibition effect on the cellular uptake of [^{14}C]-benzoic acid in Caco-2 cells with IC_{50} values of 0.05 0.44 mM. The inhibition of naproxen and ketoprofen against the membrane transport of [^{14}C]-benzoic acid appeared to be competitive with Ki of 0.22 mM and 0.38 mM, respectively. The membrane permeability of naproxen and ketoprofen was concentration dependent, implying that the cellular uptake pathway of ketoprofen and naproxen was saturable at the high concentration. Furthermore, the cellular accumulation of ketoprofen was significantly reduced in the presence of benzoic acid and L-lactic acid, two known substrates of monocarboxylic acid transporter 1 (MCT1). These results suggest that MCT1 contributes at least in part to a carrier-mediated transport of NSAIDs containing a carboxylic acid moiety across the apical membrane in Caco-2 cells.

The present study aims to investigate the intestinal absorption characteristics of ketoprofen in rats. The pharmacokinetic profile of ketoprofen was evaluated following a single p.o. administration of ketoprofen (1mg/kg) to rats in the absence and presence of benzoic acid or lactic acid (2 and 10 mg/kg), the substrates of monocarboxylic acid transporters. Pharmacokinetic profiles

of ketoprofen (1 mg/kg) were significantly altered by the concurrent use of benzoic acid or lactic acid (10 mg/kg), compared to the control (given ketoprofen alone). C_{max} and AUC of ketoprofen in the presence of benzoic acid or lactic acid (10 mg/kg) were significantly ($p<0.05$) lower than those from the control group, while there was no significant change in T_{max} and terminal plasma half-life ($T_{1/2}$) of ketoprofen. Those results suggest that ketoprofen shares a common transport pathway with benzoic acid and lactic acid during the intestinal absorption in rats.

Key words: NSAIDs, monocarboxylic acid transporter, cellular uptake, Caco-2 cell

1.In-vitro study

1-1 Introduction

The transport of monocarboxylates such as lactate and pyruvate across the plasma membrane of mammalian cells is facilitated by a family of monocarboxylate/H+ co-transporters (MCT) (Juel et al. 1999; Poole et al. 1993; Tamai et al. 1995a). So far, 14 members of MCTs have been identified but only MCT1-4 have been expressed in an active form and characterized as proton-linked monocarboxylic acid transporters (Enerson et al. 2003; Halestrap et al. 1999; Halestrap et al. 2004; Makuc et al. 2004). Of these, solely the MCT1 isoform plays a major role in the transport of various monocarboxylates across the gastrointestinal epithelia, whereas other MCT isoforms seem to be of little or no importance(Enerson et al. 2003; Halestrap et al. 1999; Orsenigo et al. 1999; Ritzhaupt et al. 1998a&b). Several studies have reported that MCT1 is located in the brush-border membranes of both the upper and lower intestines and has an important role in the intestinal absorption of pharmacologically active compounds such as β-lactam antibiotics (phenethicillin, propicillin, carindacillin etc), atorvastatin, and pravastatin (Kang et al. 1990; Li et al. 1999; Tamai et al. 1995b; Wu et al. 2000).

Many Nonsteroidal anti-inflammatory drugs (NSAIDs) have a monocarboxylic acid grouping their structures. Those NSAIDs are in general rapidly absorbed from the gastrointestinal tract, however, the mechanism of transport across the intestinal epithelia is not clear yet. Some studies proposed that the membrane transport of NSAIDs should be facilitated by MCTs (Emoto et al. 2002; Takanaga et al. 1994; Tamai et al. 1995a; Tsuji et al. 1996), while some authors suggested the pH dependent but non carrier-mediated absorption of NSAIDs (Legen et al. 2003; Takagi et al. 1998). Therefore, the transport mechanism of NSAIDs is still uncertain. Considering the clinical significance of drug-drug interactions mediated by drug transporters, it is important to evaluate the contribution of a carrier-mediated mechanism to the membrane transport of drugs. Particularly, interactions between NSAIDs and other anionic drugs including nucleoside antiviral drugs, antibiotics and hippurates may occur relatively frequent, since NSAIDs are widely used as prescription or over-the-counter drugs. Therefore, the present study aims to clarify the cellular uptake mechanism of NSAIDs, particularly the potential contribution of a carrier-mediated mechanism to the overall absorption of NSAIDs.

Caco-2 cells express the five isoforms of MCTs (MCT1 and MCT3-6), of which MCT1 is the most abundant isoform in Caco-2 cells (Hadjiagapiou et al. 2000). Therefore, in the present study, Caco-2 cell monolayer was used as an appropriate in-vitro model to examine the role of MCT1 in the transport of NSAIDs across the intestinal epithelial membrane.

1-2 Materials and Methods

Materials: diclofenac, diflunisal, naproxen, ketoprofen, benzoic acid, [^{14}C]-benzoic acid (13.1 mCi/mmol), L-lactic acid and BCA protein assay kit were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Caco-2 cells were purchased from ATCC (Rockville, MD, USA). All other chemicals were reagent grade and all solvents were HPLC grade.

Cell Cultures: Caco-2 cells were routinely maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS, 1% nonessential amino acids, 1 mM sodium pyruvate, 1% L-glutamine and penicillin (100 U/mL)/streptomycin (100 mg/mL). All cells were maintained in an atmosphere of 5% $CO_2$and 90% relative humidity at 37 ˚C.

Inhibition Studies in Caco-2 cells: Cells were seeded in 12-well culture plates at a density of 10^5 cells/cm^2. At 2-3 weeks post-seeding, the cells were washed twice with pH 6.0 uptake buffer containing 1mM $CaCl_2$, 1mM $MgCl_2$, 150 mM NaCl, 3 mM KCl, 1 mM NaH_2PO_4, 5 mM D-glucose, and 5 mM MES. Each test solution (0.1 1000 uM) containing [^{14}C]-benzoic acid (20 uM, 0.1 uCi/mL) was added to each well and incubated for 15 min. At the end of incubation, drug solution was removed and the cells were washed three times with ice-cold PBS. One milliliter of 1.5 % ice-cold Triton X solution was added to each well. After 15 min incubation, cells were harvested and the radioactivity in each sample was determined by a scintillation counter.

Uptake studies in Caco-2 cells: The studies were carried out in 6-well plates with confluent cells as described in inhibition studies. Briefly, the initial uptake rates of ketoprofen (0.5 and 2.5 mM) and naproxen (0.5 and 5 mM) were determined in pH 6.0 uptake buffer to examine the concentration dependency in their cellular accumulation. The uptake of ketoprofen (0.5 mM) was also measured in the absence and presence of inhibitors. At the end of 15 min incubation, the cells were washed three times with ice-cold PBS and ruptured directly on the plate by adding 1 mL of Milli-Q water. Cells were harvested and sonicated for 1-2 min. Trichloroacetic acid (3-5%) was added to the cell lysate, vortexed rigorously, and centrifuged for 5 min at 3000 rpm. After filtration of the supernatant through a membrane filter (0.45 μm), samples were analyzed by HPLC. The protein amount of each sample was determined with BCA protein assay kit following the manufacturer's instruction (Sigma Chemical Co., St. Louis, MO, USA).

HPLC Assay: Concentrations of ketoprofen and naproxen were determined by a HPLC assay described as follows. Naproxen was used as the internal standard for the assay of ketoprofen and ketoprofen as the internal standard for the naproxen assay. The chromatographic system was consisted of a pump (LC-10AD), an automatic injector (SIL-10A) and a UV detector (SPD-10A) (Shimadzu Scientific Instruments, Tokyo, Japan). An octadecylsilane column (Gemini C18, 4.6 × 250 mm, 5μm; Phenomenex, Torrance, CA, USA) was eluted with a mobile phase consisting of 0.01M phosphate buffer (pH 7.0):acetonitrile (75:25, v/v %) at a flow rate of 1.0 mL/min. Ketoprofen and naproxen were monitored at 258 and 229 nm, respectively. The calibration curve from the standard samples was linear over the concentration range of 0.01 μg/mL to 10 μg/mL. The limit of detection was 0.01 μg/mL.

Data Analysis:

(A) Estimate of IC_{50}: $IC_{50}$is defined as the drug concentration to show the 50% inhibition on the uptake of benzoic acid. As described by De Lean et al. [17], it was determined from the nonlinear regression of a dose-response curve by using the Sigma Plot® 9.0 (Systat Software Inc., Point Richmond, CA, USA).

(B) Estimate of Permeability**:** Permeability coefficient (P_{app}) was calculated from the linear portion of an uptake versus time plot using the follow equation:

$P_{app} = (dm/dt) * 1/(A \cdot C_0)$

where A is diffusion area (cm^2), C_0 is the initial concentration and dm/dt is the initial uptake rate.

Statistical analysis: All the means are presented with their standard deviation. Statistical analysis was performed using a one-way ANOVA, followed by a posteriori testing with the use of the Dunnett correction. A *P* value < 0.05 was considered statistically significant.

1-3 Results and Discussion

Inhibition Studies on the uptake of benzoic acid in Caco-2 cells:

Previous studies have reported that the transport of benzoic acid and L-lactic acids were facilitated by the MCT1 (Juel et al. 1999; Poole et al. 1993; Tamai et al. 1995). Therefore, in the present study, benzoic acid and L-lactic acids were selected as the representative substrates for MCT1 to examine the interaction between NSAIDs and MCT1 in Caco-2 cells. Four structurally diverse NSAIDs such as diclofenac, diflunisal, ketoprofen and naproxen (Fig.1-1) were compared in Caco-2 cells with respect to their inhibitory effect on the uptake of benzoic acid. As summarized in (Table 1-1) and (Fig. 1-2), all the tested drugs exhibited the strong inhibition effect on the uptake of [^{14}C]-benzoic acid with an IC_{50} value of 0.05 mM to 0.44 mM. These results supported the previous report by Konishi et al. (2002), suggesting that monoanionic carboxyl group and an unpolar side chain or aromatic hydrophobic portion may be necessary to be recognized by MCTs. Diclofenac and diflunisal appeared to be more potent inhibitors against the uptake of benzoic acid than ketoprofen and naproxen, implying that the inhibitory potency might be influenced by the type of a carboxylic acid. Acetic acid side chain (diclofenac) seemed to be more favorable for the interaction with MCT1 than a propionic acid side chain (naproxen and ketoprofen), suggesting that a methyl substituent in the immediate vicinity of a carboxylic acid group may decrease the affinity to MCT1. Furthermore, direct attachment of a carboxyl moiety to the hydrophobic aromatic ring (diflunisal) seemed to enhance the binding affinity to the monocarboxylic acid transporters.

Kinetic analysis using Lineweaver-Burk plots was also performed to clarify the inhibition mode of naproxen and ketoprofen on the accumulation of benzoic acid in Caco-2 cells. As illustrated in (Fig. 1-3), both naproxen and ketoprofen inhibited the cellular uptake of benzoic acid in a competitive manner with the inhibition constant values (Ki) of 0.22 0.05 mM and 0.38 0.07 mM, respectively.

Collectively, NSAIDs containing a carboxylic acid moiety such as diflunisal, diclofenac, naproxen and ketoprofen were able to interact with monocarboxylic acid transporter 1, a transport system of benzoic acid.

Cellular uptake studies in Caco-2 cells: To evaluate the potential contribution of a carrier-mediated transport mechanism to the cellular uptake of monocarboxylic acid type NSAIDs, the concentration dependency in the membrane transport of naproxen and

ketoprofen was examined in Caco-2 cells. As summarized in Table1-2, the apparent permeability of naproxen and ketoprofen decreased significantly ($p<0.05$) as a drug concentration increased by 5-10 folds, implying that the cellular uptake pathway of ketoprofen and naproxen was saturable at the high concentration. Furthermore, the transport of ketoprofen across the apical membrane of the Caco-2 cell monolayers was markedly inhibited by the presence of benzoic acid or L-lactic acid, supporting that the cellular uptake of ketoprofen shares a common transport pathway at least partially with benzoic acid and L-lactic acid (Fig.1-4). This finding is contradictive with the previous reports by Legen et al. (2003), while others (Emoto et al. 2002; Ogihara et al. 1996; Takanaga et al. 1994; Tamai et al. 1995) are more supportive to our findings. By using the excised rat jejunal segment mounted in side-by-side diffusion cells, Legen et al. (2003) reported that ketoprofen transport was not saturable over the concentration range of 0.125 to 5 mM and was not inhibited by benzoic acid or L-lactic acid. The explanation on this discrepancy is not clear yet. Maybe, the Km values for MCT1-mediated drug transport is much higher in the excised rat jejunal model than that in Caco-2 cells and thus, drug concentrations tested in their experiments might not be appropriate to observe the saturation nor significant inhibition on the carrier-mediated transport of ketoprofen. Discrepancy in the results obtained from the different in-vitro settings should be further clarified based on the in-vivo relevance of in-vitro findings. Therefore, the quantitative evaluation on the contribution of different mechanisms to the whole process of the NSAIDs absorption need to be undertaken in vivo in future studies.

In conclusion, monocarboxylic acid transporter1 (MCT1) contributes at least in part to the transport of NSAIDs containing a carboxylic acid moiety across the apical membrane in Caco-2 cells.

2.In-vivo study

2-1 Introduction

ManyNonsteroidal anti-inflammatory drugs (NSAIDs) have a monocarboxylic acid group in their structure. Those weak organic acids are in general rapidly absorbed from the gastrointestinal tract, however, the mechanism of transport across the intestinal epithelia is still uncertain. Previous studies have demonstrated that under the inwardly directed proton gradient across the brush border membrane, several monocarboxylic acids such as benzoic acid, atorvastatin, pravastatin and carindacillin could be transported across the intestinal epithelia by proton/monocarboxylate co-transporters (MCTs) [1-4]. Given that MCTs are widely distributed throughout various mammalian tissues [5-7] and numerous drugs contain a carboxyl group making these compounds potential substrates for MCTs, they may have an important role in the transport of various exogenous compounds. So far, 14 members of MCTs have been identified but only MCT1-4 have been expressed in an active form and characterized as proton-linked MCTs [8-11]. Of these, solely the MCT1 isoform plays a major role in the transport of various monocarboxylates across the gastrointestinal epithelia, whereas other MCT isoforms seem to be of little or no importance [8, 9, 12-14]. Previous studies have reported that NSAIDs could interact with MCTs expressed in vitro and proposed the MCT-mediated transport of NSAIDs across the intestinal epithelia [15-19]. In contrast, Takagi et al. suggested that the absorption of monocarboxylic acid type NSAIDs could be facilitated by the inwardly directed proton gradient across the brush border membrane, which is maintained by the acidic microclimate on the mucosal surface [20]. Legend et al. also supported this pH-dependent but non-MCT1 mediated pathway for the transport of ketoprofen [21-22].

So far, no direct evaluation of those proposed mechanisms has been undertaken in vivo and it is not clear yet which mechanism is a major contributor for the rapid absorption of NSAIDs from the intestinal lumen in vivo. Therefore, the present study aims to investigate the intestinal absorption characteristics of ketoprofen in rats.

2-2 Materials and Methods

Materials: Ketoprofen, naproxen, benzoic acid, and lactic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were reagent grade and all solvents were HPLC grade.

Animal Studies: All animal studies were performed in accord with the Principles for Biomedical Research Involving Animals developed by the Council for International Organizations of Medical Sciences and the experimental protocols were approved by the animal care committee of Chosun University.

Male Sprague-Dawley rats weighing 280-320 g were obtained from Samtako Bio Co., Ltd (Osan, Korea). At the experiment, rats were divided into six groups, comprising 4 rats per each group. Group 1-5 were given 1 mg/kg of ketoprofen (PO, dosing volume: 1 mL) with either benzoic acid (2 or 10 mg/kg), lactic acid (2 or 10 mg/kg), or no concomitant treatment (control). Group 6 was given 10 mg/kg of ketoprofen. Blood samples were collected from the right femoral artery at 0, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hr following a ketoprofen administration and then centrifuged at 3,000 rpm for 10 min to obtain the plasma for the HPLC assay. All samples were stored at - 70℃ until analyzed.

HPLC Assay: The concentrations of ketoprofen were determined by a HPLC assay described as follows. Naproxen was used as the internal standard for the assay of ketoprofen. The extraction residue containing internal standard was reconstituted with 100 μL of mobile phase and then a 50 μL of aliquots was injected directly into the HPLC system. The chromatographic system was consisted of a pump (LC-10AD), an automatic injector (SIL-10A) and a UV detector (SPD-10A) (Shimadzu Scientific Instruments, Japan). An octadecylsilane column (Gemini C18, 4.6 × 250 mm, 5μm; Phenomenex, Torrance, CA, USA) was eluted with a mobile phase consisting of 0.01M phosphate buffer (pH 7.0) : acetonitrile (75:25 v/v %) at a flow rate of 1.0 mL/min. Ketoprofen was monitored at 258 nm. The calibration curve from the standard samples was linear over the concentration range of 0.01 μg/mL to 10 μg/mL. The limit of detection was 0.01 μg/mL.

Data Analysis

Pharmacokinetic Analysis: Non-compartmental pharmacokinetic analysis was performed using Kinetica-4.3 (Inna Phase Corp., Philadelphia, PA, USA). The area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal method. Maximum plasma concentration (C_{max}) and the time to reach the maximum plasma concentration (T_{max}) were read directly from the plasma concentration-time data. The terminal elimination rate constant (λz) was estimated from the slope of the terminal phase of the log plasma concentration-time points fitted by the method of least-squares, and then the terminal elimination half-life ($T_{1/2}$) was calculated as $0.693/ \lambda z$.

Statistical analysis: All the means are presented with their standard deviation. The pharmacokinetic parameters were compared with a one-way ANOVA, followed by a posteriori testing with the use of the Dunnett correction. A P value < 0.05 was considered statistically significant.

2-3 Results and Discussion

There is increasing evidence suggesting that clinically important drug interactions can be caused by the modulation of drug transporters. Therefore, it is important to evaluate the potential contribution of a carrier-mediated mechanism to the intestinal absorption of drugs, particularly for widely used drugs either as prescription or over-the-counter drugs. Among NSAIDs, ketoprofen is rapidly absorbed from gastrointestinal tract with the bioavailability of 90 98 % [23, 24] but the intestinal absorption mechanism of ketoprofen is still controversial. In our previous in-vitro studies, the cellular uptake of ketoprofen appeared to be carrier-mediated and substantially inhibited by the presence of benzoic acid or L-lactic acid, the representative substrates of MCT1 [15]. However, this finding is contradictive with the previous reports by Legen et al. [22]. By using the excised rat jejunal segment mounted in side-by-side diffusion cells, Legen et al. [22] reported that ketoprofen transport was not saturable over the concentration range of 0.125 to 5 mM and was not inhibited by benzoic acid or lactic acid. This discrepancy may be explained by that the Km values for MCT-mediated drug transport is much higher in the excised rat jejunal model than that in the in-vitro cells and thus, drug concentrations tested in their experiments might not be appropriate to observe the saturation nor significant inhibition on the carrier-mediated transport of ketoprofen. The discrepancy in the results obtained from the different in-vitro settings should be further clarified based on the in-vivo relevance of in-vitro findings. Therefore, in the present study, the mean plasma concentration-time profiles of ketoprofen in the presence and absence of benzoic acid or lactic acid were evaluated in rats. The mean pharmacokinetic parameters of ketoprofen were summarized in (Table2-1).

The pharmacokinetics of ketoprofen following an oral administration to rats was linear over the dose range of 1 mg/kg to 10 mg/kg (data not shown) and comparable to those from the previous studies [25, 26]. As summarized in Table2-1, the concurrent use of benzoic acid or lactic acid at 2-mg/kg did not affect the pharmacokinetics of ketoprofen. However, at 10-mg/kg dose, the presence of benzoic acid or lactic acid significantly altered the systemic exposure of ketoprofen in rats, compared to the control given ketoprofen alone (Fig2-1). C_{max} of ketoprofen decreased by about 35% and AUC was reduced by two to three fold ($p<0.05$) in the presence of benzoic acid or lactic acid at 10-mg/kg, while there was no significant change in T_{max} and terminal plasma half-life ($T_{1/2}$), implying that the decrease in the systemic exposure of ketoprofen under the co-administration of benzoic acid or lactic acid could be accounted for by the reduction in the

intestinal absorption of ketoprofen. Therefore, ketoprofen appeared to share the intestinal absorption pathway with benzoic acid and lactic acid. Considering that the transport of benzoic acid and lactic acids were facilitated by the MCT1, those results are consistent with the previous in-vitro studies indicating that NSAIDs containing a monocarboxylic acid moiety could interact with MCT1 [15-19]. In contrast, Legen et al. have reported that ketoprofen transport was not saturable over the concentration range of 0.125 to 5 mM and was not inhibited by benzoic acid or lactic acid [21, 22]. Considering that the inhibition effect of benzoic acid and lactic acid on the intestinal absorption of ketoprofen was dose dependent in rats (Table2-1), drug (or inhibitor) concentrations tested in their experiments might not be appropriate to observe the saturation nor significant inhibition on the carrier-mediated pathway for ketoprofen.

In summary, the intestinal absorption of ketoprofen decreased in the presence of benzoic acid or lactic acid, suggesting that ketoprofen shares a common transport pathway with benzoic acid and lactic acid across the intestinal membrane in rats.

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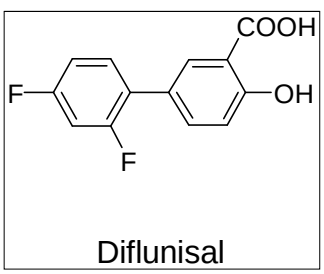
Table1-1: Inhibition on the uptake of [^{14}C]-benzoic acid in Caco-2 cells (Mean±SD, n=6)

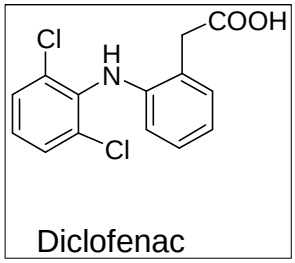
Drugs	IC_{50} (mM)
Diclofenac	0.10 ± 0.02
Diflunisal	0.05 ± 0.01
Ketoprofen	0.44 ± 0.09
Naproxen	0.25 ± 0.03

Table1-2: Concentration dependency in the transport of ketoprofen and naproxen across the apical membrane of Caco-2 cells
(Mean±SD, n = 6)

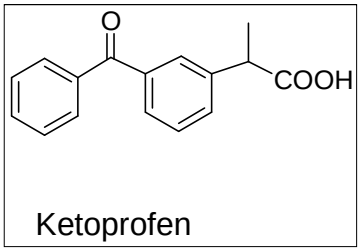
	Ketoprofen		Naproxen	
	0.5 mM	2.5 mM	0.5 mM	5 mM
Papp ($\times 10^{-6}$, cm/sec)	3.9 ± 0.29	1.6 ± 0.54	4.9 ± 0.61	1.5 ± 1.38



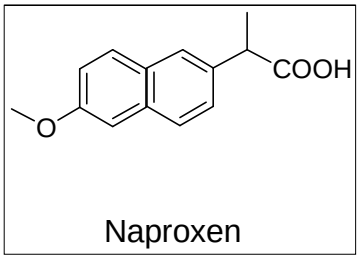
Diflunisal



Diclofenac



Ketoprofen



Naproxen

Fig.1-1: Structures of diflunisal, diclofenac, ketoprofen and naproxen

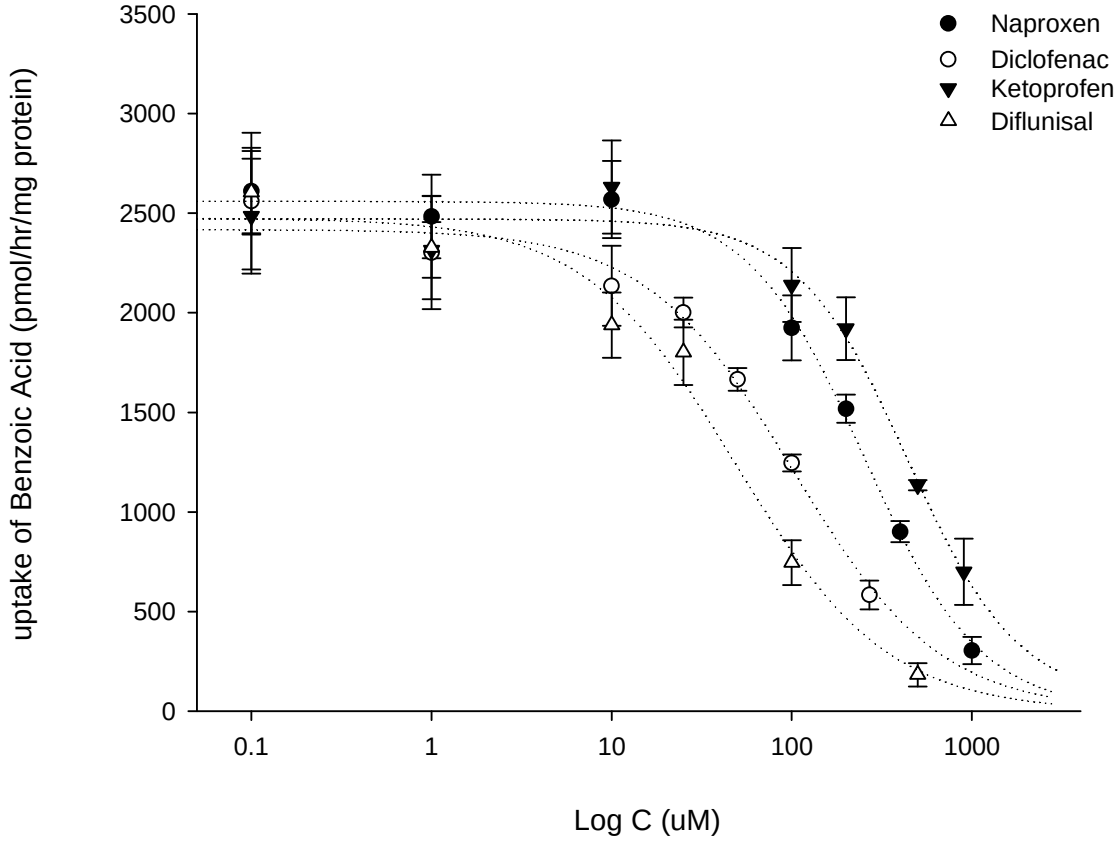
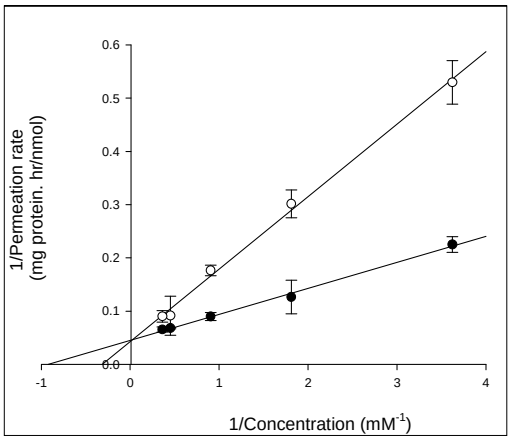


Fig.1-2: Inhibition effect of NSAIDs on the uptake of [^{14}C]-Benzoic acid in Caco-2 cells (Mean±SD, n = 6)

(A)



(B)

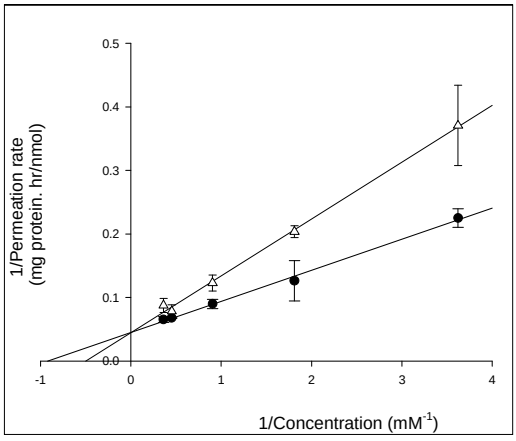


Fig.1-3: Lineweaver-Burk Plots for the transport of benzoic acid across Caco-2 cell monolayers (Mean±SD, n = 6). The transport was measured in the absence (●) and presence of 0.5 mM naproxen. (o, panel A) or 0.5 mM ketoprofen (△, panel B).

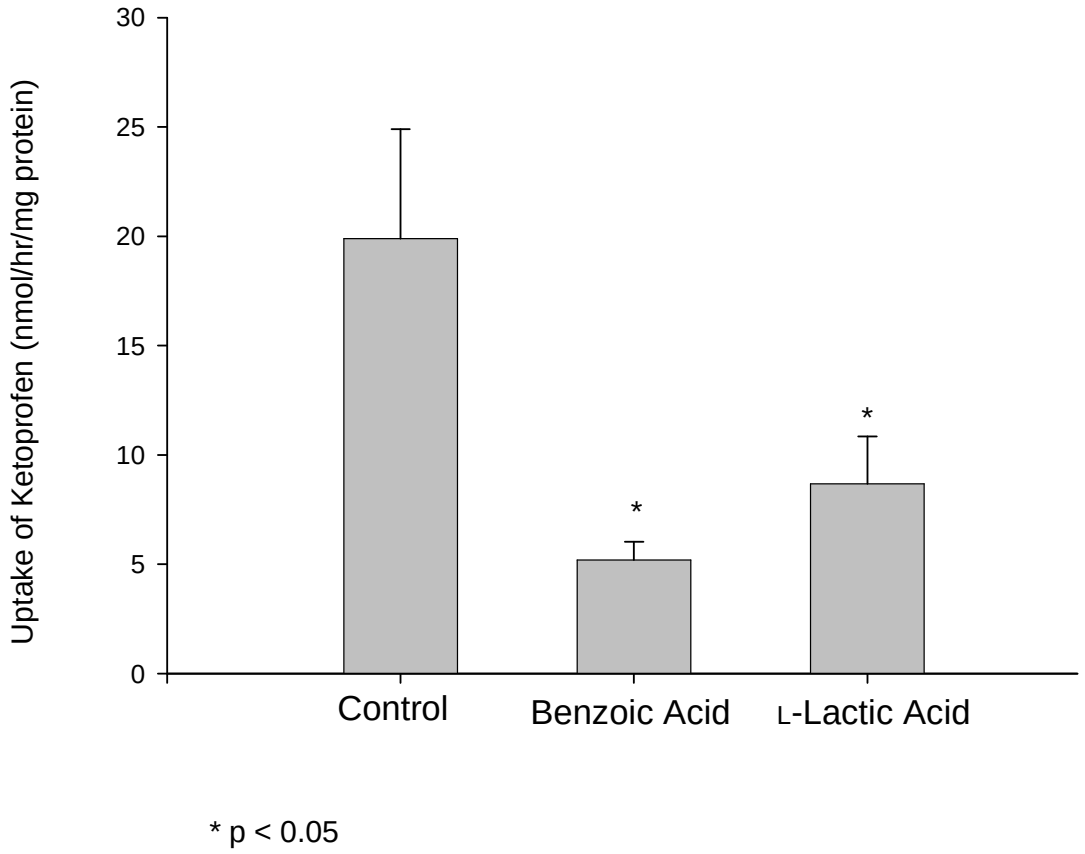


Fig.1-4: Cellular uptake of ketoprofen (0.5 mM) in the absence and presence of benzoic acid (2 mM) or L-lactic acid (2 mM) (Mean±SD, n = 6)

Table2-1. Mean pharmacokinetic parameters of ketoprofen following an oral administration of ketoprofen (1 mg/kg) to rats in the presence and absence of benzoic acid or lactic acid (Mean ± SD, n = 4)

Parameters	Tmax (hr)	Cmax (g/mL)	AUC (g/hr/mL)	$T_{1/2}$ (hr)
Ketoprofen (Control)	0.42±0.29	6.12±1.02	26.5±5.42	5.4±0.3
Ketoprofen with				
Benzoic acid (2 mg/kg)	0.25	7.59±1.54	27.1±10.3	5.9±0.4
Lactic acid (2 mg/kg)	0.25	6.93±2.35	30.4±8.29	6.6±0.7
Benzoic acid (10 mg/kg)	0.25	4.06±1.04*	13.0±2.13*	5.0±0.3
Lactic acid (10 mg/kg)	0.25	3.85±1.34*	8.49±1.23*	4.4±1.2

*: $p < 0.05$, significant difference compared to the control (given ketoprofen alone)

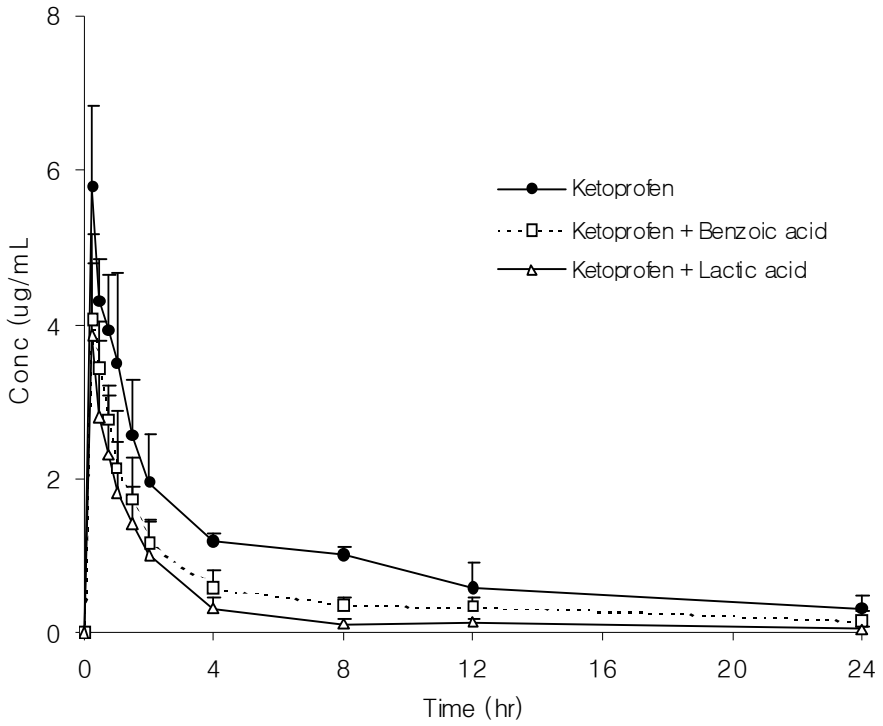


Figure2-1. Mean pharmacokinetic profiles of ketoprofen following an oral administration of ketoprofen (1mg/kg) to rats in the presence and absenceof benzoic acid or lactic acid (10 mg/kg) (Mean + SD, n = 4)