BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **27**: 17–21 (2006)
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI**: 10.1002/bdd.479

Intestinal Absorption Characteristics of Ketoprofen in Rats

Jun-Shik Choi, Ming Ji Jin and Hyo-Kyung Han*
College of Pharmacy, Cho-Sun University, Seosuk-dong, Gwangju, Korea

ABSTRACT: 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 (1 mg/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. The pharmacokinetic profiles of ketoprofen (1 mg/kg) were significantly altered by the concurrent use of benzoic acid or lactic acid (10 mg/kg), compared with the control (given ketoprofen alone). The 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 the terminal plasma half-life ($T_{1/2}$) of ketoprofen. These results suggest that ketoprofen shares a common transport pathway with benzoic acid and lactic acid during the intestinal absorption in rats. Copyright © 2005 John Wiley & Sons, Ltd.

Key words: ketoprofen; absorption; monocarboxylic acid transporter; benzoic acid; lactic acid

Introduction

Many nonsteroidal 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

*Correspondence to: College of Pharmacy, Cho Sun University, 375 Seosuk Dong, Dong-Gu, Gwangju, Korea.
E-mail: hkhan@chosun.ac.kr

Received 27 May 2005
Accepted 19 August 2005

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.

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 accordance 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). For the experiment, the rats were divided into six groups, comprising four rats per group. Group 1–5 were given 1 mg/kg of ketoprofen (p.o., 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 h following a ketoprofen administration and then centrifuged at 3000 rpm for 10 min to obtain the plasma for the HPLC assay. All samples were stored at -70°C until analysed.

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 aliquot was injected directly into the HPLC system. The chromatographic system 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, 5 µm; Phenomenex, Torrance, CA, USA) was eluted with a mobile phase consisting of 0.01 M 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 0.01–10 µg/ml. The limit of detection was 0.01 µg/ml.

Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was performed using Kinetica-4.3 (InnaPhase Corp., Philadelphia, PA, USA). The area under the plasma concentration-time curve (*AUC*) was calculated using the linear trapezoidal method. The 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.

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. Of the NSAIDs, ketoprofen is rapidly absorbed from the

gastrointestinal tract with a 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 contradicts 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 0.125–5 mM and was not inhibited by benzoic acid or lactic acid. This discrepancy may be explained by the fact that the K_m values for MCT-mediated drug transport is much higher in the excised rat jejunal model than that in the *in vitro* cells and thus, the 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 the *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 evaluated in rats. The mean pharmacokinetic parameters of ketoprofen are summarized in Table 1.

The pharmacokinetics of ketoprofen following an oral administration to rats was linear over the dose range 1–10 mg/kg (data not shown) and comparable to those from the previous studies [25,26]. As summarized in Table 1, the concurrent use of benzoic acid or lactic acid at 2 mg/kg did not affect the pharmacokinetics of ketoprofen. However, at a 10 mg/kg dose, the presence of benzoic acid or lactic acid significantly altered the systemic exposure of ketoprofen in rats, compared with the control given ketoprofen alone (Figure 1). The C_{max} of ketoprofen decreased by about 35% and the *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 the 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 a 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

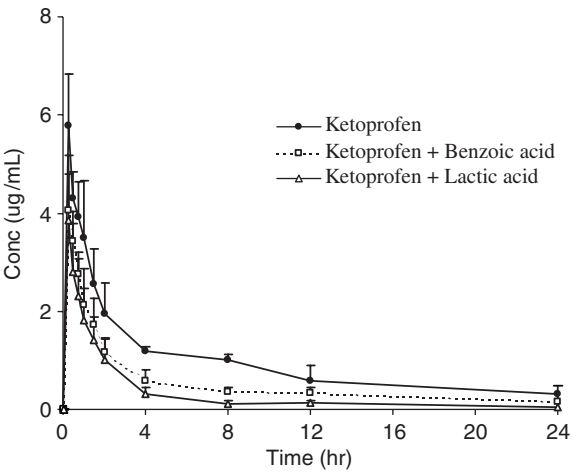

Figure 1. Mean pharmacokinetic profiles of ketoprofen following an oral administration of ketoprofen (1 mg/kg) to rats in the presence and absence of benzoic acid or lactic acid (10 mg/kg) (mean+SD, $n = 4$)

Table 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, $n = 4$)

Parameter	T_{max} (h)	C_{max} (μg/ml)	*AUC* (μg/h/ml)	$T_{1/2}$ (h)
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[a]	13.0 ± 2.13[a]	5.0 ± 0.3
Lactic acid (10 mg/kg)	0.25	3.85 ± 1.34[a]	8.49 ± 1.23[a]	4.4 ± 1.2

[a] $p < 0.05$, significant difference compared with the control (given ketoprofen alone).

acid and lactic acid was facilitated by MCT1, these 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 0.125–5 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 (Table 1), the 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.

References

1. Li YH, Ito K, Tsuda Y, Kohda R, Yamada H, Itoh T. Mechanism of intestinal absorption of an orally active b-lactam prodrug: uptake and transport of carindacillin in caco-2 cells. *J Pharmacol Exp Ther* 1999; **290**: 958–964.
2. Tamai I, Takanaga H, Maeda H, Ogihara T, Yoneda M, Tsuji A. Proton-cotransport of pravastatin across intestinal brush-border membrane. *Pharm Res* 1995; **12**: 1727–1732.
3. Tsuji A, Takanaga H, Tamai I, Terasaki T. Transcellular transport of benzoic acid across caco-2 cells by a pH-dependent and carrier-mediated transport mechanism. *Pharm Res* 1994; **11**: 30–37.
4. Wu X, Whitfield LR, Stewart BH. Atorvastatin transport in the caco-2 cell model: contributions of P-glycoprotein and the proton-monocarboxylic acid co-transporter. *Pharm Res* 2000; **17**: 209–215.
5. Garcia CK, Brown MS, Pathak RK, Goldstein JL. cDNA cloning of MCT2, a second monocarboxylate transporter expressed in different cells than MCT1. *J Biol Chem* 1995; **270**: 1843–1849.
6. Juel C, Halestrap AP. Lactate transport in skeletal muscle—role and regulation of the monocarboxylate transporter. *J Physiol* 1999; **517**: 633–642.
7. Price NT, Jackson VN, Halestrap AP. Cloning and sequencing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past. *Biochem J* 1998; **329**: 321–328.
8. Enerson BE, Drewes LR. Molecular features, regulation, and function of monocarboxylate transporters: Implications for drug delivery. *J Pharm Sci* 2003; **92**: 1531–1544.
9. Halestrap AP, Price NT. The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. *Biochem J* 1999; **343**: 281–299.
10. Halestrap AP, Meredith D. The SLC16 gene family—from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. *Pflugers Arch* 2004; **447**: 619–628.
11. Makuc J, Cappellaro C, Boles E. Co-expression of a mammalian accessory trafficking protein enables functional expression of the rat MCT1 monocarboxylate transporter in *Saccharomyces cerevisiae*. *FEMS Yeast Res* 2004; **4**: 795–801.
12. Orsenigo MN, Tosco M, Bazzini C, Laforenza U, Faelli A. A monocarboxylate transporter MCT1 is located at the basolateral pole of rat jejunum. *Exp Physiol* 1999; **84**: 1033–1042.
13. Ritzhaupt A, Ellis A, Hosie KB, Shirazi-Beechey SP. The characterization of butyrate transport across pig and human colonic luminal membrane. *J Physiol* 1998; **507**: 819–830.
14. Ritzhaupt A, Wood IS, Ellis A, Hosie KB, Shirazi-Beechey SP. Identification and characterization of a monocarboxylate transporter (MCT1) in pig and human colon: its potential to transport L-lactate as well as butyrate. *J Physiol* 1998; **513**: 719–732.
15. Choi JS, Jin MJ, Han H-K. Role of monocarboxylic acid transporters in the cellular uptake of NSAIDs. *J Pharm Pharmacol* 2005; **57**: 1185–1189.
16. Takanaga H, Tamai I, Tsuji A. pH-dependent and carrier-mediated transport of salicylic acid across caco-2 cells. *J Pharm Pharmacol* 1994; **46**: 567–570.
17. Tamai I, Takanaga H, Maeda H, *et al*. Participation of a proton-cotransporter, MCT1, in the intestinal transport of monocarboxylic acids. *Biochem Biophys Res Commun* 1995; **214**: 482–489.
18. Tamai I, Sai Y, Ono A, *et al*. Immunohistochemical and functional characterization of pH-dependent intestinal absorption of weak organic acids by the monocarboxylic acid transporter MCT1. *J Pharm Pharmacol* 1999; **51**: 1113–1121.
19. Tsuji A, Tamai I. Carrier-mediated intestinal transport of drugs. *Pharm Res* 1996; **13**: 963–977.
20. Takagi M, Taki Y, Sakane T, *et al*. 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* 1998; **285**: 1175–1180.
21. Legen I, Zakelj S, Kristl A. Polarised transport of monocarboxylic acid type drugs across rat jejunum *in vitro*: the effect of mucolysis and ATP-depletion. *Int J Pharm* 2003; **256**: 161–166.
22. Legen I, Kristl A. pH and energy dependent transport of ketoprofen across rat jejunum *in vitro*. *Eur J Pharm Biopharm* 2003; **56**: 87–94.

23. Jamali F, Brocks DR. Clinical pharmacokinetics of ketoprofen and its enantiomers. *Clin Pharmacokinet* 1990; **19**: 197–217.
24. Palazzini E, Galli G, Babbini M. Pharmacokinetic evaluation of conventional and controlled-release product of naproxen. *Drugs Exp Clin Res* 1990; **16**: 243–247.
25. Satterwhite JH, Boudinot FD. Pharmacokinetics of ketoprofen in rats: effect of age and dose. *Biopharm Drug Dispo* 1992; **13**: 197–212.
26. Foster RT, Jamali F. Stereoselective pharmacokinetics of ketoprofen in the rat: Influence of route of administration. *Drug Metab Dispos* 1988; **16**: 623–626.