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SHORT COMMUNICATION

**Shirou Itagaki · Yoshinobu Sumi · Soji Shimamoto
Tatsuya Itoh · Takeshi Hirano · Isao Takemoto
Ken Iseki**

Secretory transport of irinotecan metabolite SN-38 across isolated intestinal tissue

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Abstract *Purpose*: The purpose of this study was to investigate the transport mechanisms of transporters that contribute to the intestinal efflux of 7-ethyl-10-hydroxycamptothecin (SN-38). *Methods*: The intestinal transport of SN-38 was studied in rat intestinal tissue mounted in Ussing chambers. *Results*: In the ileum, the level of transport from the serosal layer to the mucosal layer was significantly greater than that from the mucosal layer to the serosal layer, whereas a significant difference was not observed in the jejunum. This secretory transport required metabolic energy and was diminished by sulfobromophthalein. However, mitoxantrone, an inhibitor of breast cancer resistance protein (BCRP), did not affect the ileal secretion of SN-38. *Conclusions*: The results suggest that a specific transport system, which is distinct from BCRP, plays a major role in the secretion of SN-38 and that this secretory transport system predominantly exists in the ileum.

Keywords Irinotecan · BCRP · Ileum · Transporter

Introduction

Irinotecan hydrochloride, 7-Ethyl-10-(4-[1-piperidino]-1-piperidino)-carbonyloxycamptothecin (CPT-11) is a synthetic derivative of the plant alkaloid camptothecin, which has demonstrated pronounced antitumor activity [9]. Unlike other clinically used camptothecin analogs, CPT-11 is a prodrug with very little inherent antitumor activity that needs to be hydrolyzed by a carboxylesterase to form the active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38) [17]. SN-38 subsequently undergoes glucuronic acid conjugation to form the corresponding glucuronide, SN-38 glucuronide (SN38-Glu) [4].

The major dose-limiting toxicity after administration of CPT-11 is severe diarrhea that is often unresponsive to common antidiarrheal agents [1]. There is no generally accepted prophylactic treatment for the delayed-type diarrhea. Many pharmacokinetic analyses in humans have been performed to predict the incidence of delayed-type diarrhea. Several proposed mechanisms of this diarrhea involve biliary excretion of CPT-11 and/or its metabolites [8, 13]. It has been suggested that beta-glucuronidase derived from enterobacteria may play a major role in the development of CPT-11-induced diarrhea by mediating hydrolysis of SN-38-Glu to form active SN-38, and consequently it impairs the gut [20].

Recently, Arimori et al. [3] have reported that significant proportions of CPT-11 and SN-38 are excreted into the gastrointestinal lumen not only via the biliary route but also via the intestinal membrane route after intravenous dosing of CPT-11 in rats. They also suggested that the gastrointestinal impairment induced by CPT-11 might also occur as a consequence of the significant secretion of CPT-11 via the intestinal membrane in addition to the above-mentioned mechanism. Intestinal excretion as well as biliary excretion should therefore be taken into consideration. However, little is known about the transport mechanisms or transporters that contribute to the intestinal secretion of CPT-11 and SN-38.

The aim of this study was to characterize the efflux transport system for the active metabolite SN-38. Transport of SN-38 was investigated using the Ussing chamber method.

S. Itagaki · Y. Sumi · S. Shimamoto · T. Hirano · K. Iseki (✉)
Department of Clinical Pharmaceutics and Therapeutics,
Graduate School of Pharmaceutical Sciences,
Hokkaido University, Kita 12-jo, Nishi 6-chome,
Kita-ku, Sapporo 060-0812, Japan
E-mail: ken-i@pharm.hokudai.ac.jp
Tel.: +81-11-7063770
Fax: +81-11-7063770

T. Itoh · I. Takemoto
Department of Pharmacy, Sapporo Social Insurance
General Hospital, Chuo 2-jo, 6-chome,
Atsubetsu-ku, Sapporo 004-8618, Japan

Materials and methods

Chemicals

All chemicals and reagents used were of analytical grade. Sulfobromophthalein (BSP), probenecid and mitoxantrone were obtained from Sigma (St. Louis, Mo.). Verapamil and *p*-aminohippuric acid (PAH) were purchased from Wako Pure Chemical Company (Osaka, Japan). SN-38 was kindly supplied by Daiichi Pharmaceutical Company (Tokyo, Japan). SN-38 was dissolved in DMSO (2% w/v final concentration) due to its hydrophobic property and poor solubility in water. The upper limit of SN-38 concentration was 25 μ*M*.

Animals

Male Wistar rats, aged 6–7 weeks (300–350 g in weight), were obtained from NRC Haruna (Gunma, Japan). The housing conditions were as described previously [10]. The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the ''Guide for the Care and Use of Laboratory Animals''.

Transport experiments

Transport experiments were carried out as described in a previous report with some modification [18]. The intestine was quickly removed and the longitudinal muscle layer was carefully stripped off with scissors. Prepared intestinal sheets were filled with Hanks' balanced salt sodium (HBSS) buffer (137 m*M* NaCl, 5.4 m*M* KCl, 1.0 m*M* $CaCl_2$, 0.8 m*M* $MgCl_2$, 0.4 m*M* KH_2PO_4, 0.3 m*M* NaH_2PO_4 and 25 m*M* D-glucose). The pH of the buffer was adjusted at 6.0 or 7.4 with 1 *N* HCl or NaOH. The prepared intestinal sheets were mounted between two Ussing-type diffusion chambers (Corning Costar Corporation, Cambridge, Mass.) that provided an exposed area of 1.78 cm^2. HBSS buffer was added to the chambers of the mucosal and serosal sides. The volume of bathing solution on each side was 3 ml, and the solution temperature was maintained at 37°C in a water-jacketed reservoir. The solution was bubbled with a 95:5 mixture of O_2/CO_2 before and during the transport experiment. The buffer solution in one of the chambers contained a substrate. Inhibitors were added to both chambers. Samples of 0.3 ml were taken from the receptor side at 15, 30, 45, 60, 90 and 120 min after incubation. After centrifugation of the sample (5000 *g* for 2 min), a part of the upper aqueous layer (100 μl) was transferred to a fresh tube and 100 μl of 50 m*M* monobasic potassium phosphate (pH 2.5) was added. After vortexing briefly, the sample was left overnight at 37°C.

Analytical procedures

An HPLC system equipped with a fluorescence detector was used to determine SN-38 as previously described [11, 12]. The column was a C8 column (250×4.5 mm, 5 μm; GL Sciences). The mobile phase consisted of 50 m*M* monobasic potassium phosphate (pH 2.5) and 7 m*M* tetrabutylammonium bromide/acetonitrile (70:30, v/v). Column temperature and flow rate were 40°C and 0.8 ml/min, respectively. The fluorescence detector (F1000; Hitachi) was operated at excitation and emission wavelengths of 355 and 515 nm, respectively. The lower limit of quantitation for SN-38 was 50 pmol/ml. Intra- and interday precision (coefficient of variation, CV) did not exceed 10%. The apparent permeability coefficient (P_{app}) expressed in centimeters per second was obtained using the following equation:

$$P_{app} = dQ/dt \bullet 1/(A \bullet C_0),$$

where dQ/dt is the linear appearance rate of mass in the receiver solution, A is the filter/cell surface area (1.78 cm^3), and C_0 is the initial concentration of SN-38 (25 μ*M*).

Statistical analysis

Statistical significance was evaluated using ANOVA followed by a post hoc test or Student's *t*-test. *P* values < 0.05 were considered significant.

Results

Permeation of SN-38 across rat small intestinal tissue

In the first part of this study, the permeation of SN-38 across the jejunum and the across the ileum were compared (Fig. 1). Serosal-to-mucosal (S-to-M) permeability coefficients of SN-38 were significantly greater than mucosal-to-serosal (M-to-S) permeability coefficients in the ileum, whereas a significant difference was not observed in the jejunum. Therefore, the ileum was used in subsequent experiments. The effect of protons on the S-to-M permeability coefficient of SN-38 in the ileum was examined. As shown in Table 1, the effect of pH was negligible. Therefore, subsequent experiments were performed at pH 7.4. The S-to-M permeability coefficient of SN-38 was reduced under ATP-depleted conditions by sodium fluoride and sodium azide.

Effects of various compounds on SN-38 secretory permeation across the rat ileum

To clarify the characteristics of transporters responsible for the secretory transport of SN-38, the inhibitory

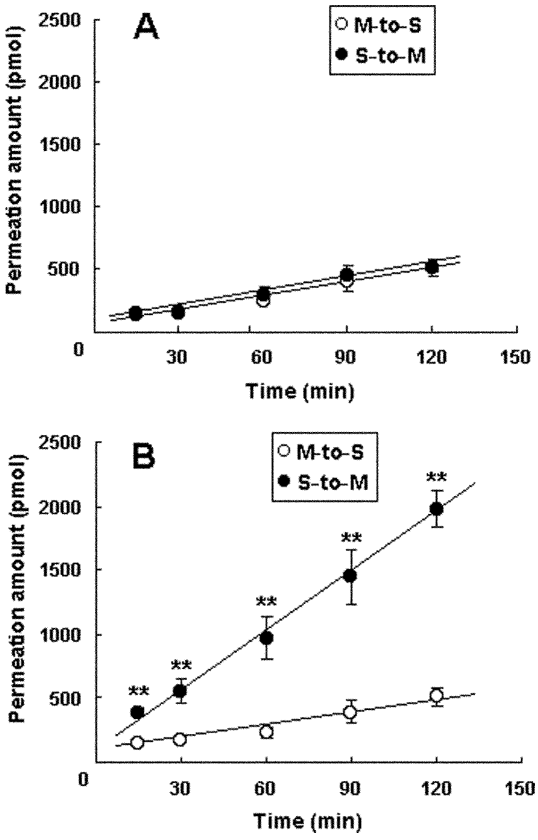

Fig. 1 Site specificity of the permeability of SN-38 in rats. Permeation of SN-38 (25 μ*M*) across the jejunum (**a**) and ileum (**b**) was evaluated by the Ussing chamber method. The experimental solution was adjusted to pH 7.4, and the temperature was maintained at 37°C. Each point represents the mean with SD of four determinations. $^{**}P < 0.01$, vs mucosal-to-serosal transport

effects of some compounds known to be inhibitors of a number of active transport systems on the transport of SN-38 in the ileum were studied. The effect of mitoxantrone, an inhibitor of breast cancer resistance protein (BCRP/*alcgz*) on the S-to-M permeability coefficient of SN-38 was examined [6]. As shown in Table 2, no significant difference was observed in the presence of mitoxantrone. It has been reported that BSP inhibits an intestinal organic anion transport system that is distinct from either P-glycoprotein (P-gp/*abcb1*) or multidrug resistance-associated protein 2 (Mrp2/*abcc2*) [19]. The effect of BSP on the permeation of SN-38 was examined. BSP significantly reduced the S-to-M permeability coefficient of SN-38. Probenecid and PAH, which are typical inhibitors of the PAH transporter, had no effect on the secretory permeation of SN-38 [18]. Moreover, verapamil, a typical inhibitor of P-gp, did not affect the secretory permeation of SN-38 [5].

Table 1 Transport characteristics of the secretion of SN-38 in rat ileal tissue. Permeation of SN-38 (25 μ*M*) was measured at 37°C for 120 min. Each value is the mean ± SD of three to four determinations

Extracellular pH (mucosal/serosal)	Inhibitors	$P_{app\ S\text{-}to\text{-}M} \times 10^6$ (cm/s)
8.5/7.4	None	5.86 ± 0.52
7.4/7.4	None	5.62 ± 0.49
6.0/7.4	None	5.71 ± 0.36
7.4/7.4	NaF/NaN_3(10 m*M*)	2.97 ± 0.72**

$^{**}P < 0.01$, vs absence of inhibitors

Table 2 Effects of various compounds on the secretion of SN-38 in rat ileal tissue. Permeation of SN-38 (25 μ*M*) was measured at 37°C for 120 min. Each value is the mean ± SD of three to four determinations

Compound	Concentration	$P_{app\ S\text{-}to\text{-}M}$ (percent of control)
Control		100
Mitoxantrone	1 m*M*	93.1 ± 6.23
BSP	200 μ*M*	53.6 ± 2.93**
PAH	1 m*M*	100 ± 4.36
Probenecid	1 m*M*	114 ± 13.7
Verapamil	1 m*M*	96.3 ± 8.17

$^{**}P < 0.01$, vs control

Discussion

Due to the unpredictable severe diarrhea observed in patients treated with CPT-11, the clinical use of this anticancer agent has remained limited [1]. It has been proposed that the severe gastrointestinal toxicity results from exposure of intestinal tissues to SN-38, due to its biliary excretion and/or deconjugation of SN-38-Glu [4, 20]. It has been reported that a significant proportion of SN-38 is excreted into the gastrointestinal lumen not only via the biliary route but also via the intestinal membrane and that intestinal exsorption of SN-38 is inhibited by cyclosporin A [2, 3]. Thus, it can be expected that reduction in the excretion of SN-38 into the gastrointestinal lumen may reduce the gastrointestinal toxicity. However, little is known about the characteristics of the intestinal SN-38 transport system.

Drug discovery, development and targeting require knowledge of transporters that play a role in the disposition of a drug and its subsequent effects [15]. In the current study, we obtained important data that provide an insight into the pathogenesis, and thus means for preventing, CPT-11-induced delayed type-diarrhea. In the first part of this study, site specificity of intestinal SN-38 secretion was examined. The results suggest that secretory transporters with a high level of activity exist in the lower region of the small intestine. To focus on the secretory mechanisms of SN-38 transport, we investigated the driving force for the SN-38 transport system. Intestinal SN-38 secretion was found to be ATP-dependent and pH-independent. These results suggest that protons or hydroxyl ions are unlikely to be the driving force for SN-38 transport.

Finally, we investigated the transport properties of intestinal SN-38 secretion. Inhibition studies were carried out using representative substrates and inhibitors of identified transporters. It has been reported that BCRP efficiently transports SN-38 in lung cancer cells in vitro [16]. BCRP is expressed in the apical side of the small intestine and colon [14]. However, the contribution of BCRP to the intestinal secretion of SN-38 has not been elucidated. Thus, the effect of BCRP inhibitor on the efflux transport of SN-38 was examined. A significant difference was not observed in the presence of mitoxantrone. The concentration of mitoxantrone was 1 m*M*, which is greater than the Km value of BCRP for SN-38 [16]. These findings suggest that the contribution of BCRP to the intestinal secretion of SN-38 is minor. On the other hand, BSP significantly reduced the efflux transport of SN-38, suggesting that a specific transport system, which is distinct from BCRP, plays a major role in the secretion of SN-38. It is thought that this secretory transport system predominantly exists in the ileum. PAH transporter and P-gp are metabolic energy-dependent transporters that have already been reported to exist in the ileum [7, 18]. In the present study, the inhibitors of these transporters did not affect SN-38 transport. These findings indicate that SN-38 efflux is not mediated by PAH transporter or P-gp.

Information on the functional characteristics of drug transporters is important for improvement in drug delivery or drug design by targeting specific transporter proteins [15]. It is thought that regulation of the functions of transporters will enable development of highly efficient drugs with ideal pharmacokinetic profiles and that approaches using intentional drug–drug interactions (positive drug interactions) may become more important in the future [21]. It is possible that coadministration of an intestinal SN-38 transporter inhibitor with CPT-11 will reduce the late-onset diarrhea that occurs during treatment with CPT-11. Thus, strategic application of intestinal SN-38 transporter inhibitors may lead to more effective oral chemotherapy with CPT-11. The expression system of transporters would be an efficient tool for screening the activity of individual transport processes. However, this transport system has not yet been elucidated at the molecular level. Further studies are needed to elucidate the mechanisms of intestinal secretion of SN-38.

In conclusion, our results suggested that a specific transport system, which is distinct from BCRP, plays a major role in the secretion of SN-38 and that this secretory transport system predominantly exists in the ileum. Inhibition of this transporter would be a useful means for reducing late-onset diarrhea.

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References

1. Abigerges D, Armand JP, Chabot GG, Da Costa L, Fadel E, Cote C, Herait P, Gandia D (1994) Irinotecan (CPT-11) high-dose escalation using intensive high-dose loperamide to control diarrhea. J Natl Cancer Inst 86:446–449
2. Arimori K, Kuroki N, Hidaka M, Iwakiri T, Yamsaki K, Okumura M, Ono H, Takamura N, Kikuchi M, Nakano M (2003) Effect of P-glycoprotein modulator, cyclosporin A, on the gastrointestinal excretion of irinotecan and its metabolite SN-38 in rats. Pharm Res 20:910–917
3. Arimori K, Kuroki N, Kumamoto A, Tanoue N, Nakano M, Kumazawa E, Tohgo A, Kikuchi M (2001) Excretion into gastrointestinal tract of irinotecan lactone and carboxylate forms and their pharmacodynamics in rodents. Pharm Res 18:814–822
4. Atsumi R, Suzuki W, Hakusui H (1991) Identification of the metabolites of irinotecan, a new derivative of camptothecin, in rat bile and its biliary excretion. Xenobiotica 21:1159–1169
5. Boesch D, Gaveriaux C, Jachez B, Pourtier-Manzanedo A, Bollinger P, Loor F (1991) In vivo circumvention of P-glycoprotein-mediated multidrug resistance of tumor cells with SDZ PSC 833. Cancer Res 51:4226–4233
6. Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, Ross DD (1998) A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci U S A 95:15665–15670
7. Fricker G, Drewe J, Huwyler J, Gutmann H, Beglinger C (1996) Relevance of p-glycoprotein for the enteral absorption of cyclosporin A: in vitro-in vivo correlation. Br J Pharmacol 118:1841–1847
8. Gupta E, Lestingi TM, Mick R, Ramirez J, Vokes EE, Ratain MJ (1994) Metabolic fate of irinotecan in humans: correlation of glucuronidation with diarrhea. Cancer Res 54:3723–3725
9. Houghton PJ, Cheshire PJ, Hallman JC, Bissery MC, Mathieu-Boue A, Houghton JA (1993) Therapeutic efficacy of the topoisomerase I inhibitor 7-ethyl-10-(4-[1-piperidino]-1-piperidino)-carbonyloxy-camptothecin against human tumor xenografts: lack of cross-resistance in vivo in tumors with acquired resistance to the topoisomerase I inhibitor 9-dimethylaminomethyl-10-hydroxycamptothecin. Cancer Res 53:2823–2829
10. Itoh T, Itagaki S, Sasaki K, Hirano T, Takemoto I, Iseki K (2004) Pharmacokinetic modulation of irinotecan metabolites by sulfobromophthalein. J Pharm Pharmacol 56:809–812
11. Itoh T, Itagaki S, Sumi Y, Hirano T, Takemoto I, Iseki K (2004) Uptake of irinotecan metabolite SN-38 by the human intestinal cell line Caco-2. Cancer Chemother Pharmacol. DOI: 10.1007/s00280-004-0937-4
12. Itoh T, Takemoto I, Itagaki S, Sasaki K, Hirano T, Iseki K (2004) Biliary excretion of irinotecan and its metabolites. J Pharm Pharm Sci 7:13–18
13. Lokiec F, Canal P, Gay C, Chatelut E, Armand JP, Roche H, Bugat R, Goncalves E, Mathieu-Boue A (1995) Pharmacokinetics of irinotecan and its metabolites in human blood, bile, and urine. Cancer Chemother Pharmacol 36:79–82
14. Maliepaard M, Scheffer GL, Faneyte IF, van Gastelen MA, Pijnenborg AC, Schinkel AH, van De Vijver MJ, Scheper RJ, Schellens JH (2001) Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. Cancer Res 61:3458–3464
15. Mizuno N, Sugiyama Y (2002) Drug transporters: their role and importance in the selection and development of new drugs. Drug Metab Pharmacokinet 17:93–108
16. Nakatomi K, Yoshikawa M, Oka M, Ikegami Y, Hayasaka S, Sano K, Shiozawa K, Kawabata S, Soda H, Ishikawa T, Tanabe S, Kohno S (2001) Transport of 7-ethyl-10-hydroxy-camptothecin (SN-38) by breast cancer resistance protein ABCG2 in human lung cancer cells. Biochem Biophys Res Commun 288:827–832

17. Narita M, Nagai E, Hagiwara H, Aburada M, Yokoi T, Kamataki T (1993) Inhibition of beta-glucuronidase by natural glucuronides of kampo medicines using glucuronide of SN-38 (7-ethyl-10-hydroxycamptothecin) as a substrate. Xenobiotica 23:5–10
18. Naruhashi K, Tamai I, Sai Y, Suzuki N, Tsuji A (2001) Secretory transport of p-aminohippuric acid across intestinal epithelial cells in Caco-2 cells and isolated intestinal tissue. J Pharm Pharmacol 53:73–81
19. Okudaira N, Komiya I, Sugiyama Y (2000) Polarized efflux of mono- and diacid metabolites of ME3229, an ester-type prodrug of a glycoprotein IIb/IIIa receptor antagonist, in rat small intestine. J Pharmacol Exp Ther 295:717–723
20. Takasuna K, Hagiwara T, Hirohashi M, Nomura M, Nagai E, Yokoi T, Kamataki T (1996) Involvement of b-glucuronidase in intestinal microflora in the intestinal toxicity of the antitumor camptothecin derivative irinotecan hydrochloride (CPT-11) in rats. Cancer Res 56:3752–3757
21. Tsuji A (2002) Transporter-mediated drug interactions. Drug Metab Pharmacokinet 17:253–274