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SHORT COMMUNICATION

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Characterization of secretory intestinal transport of the lactone form of CPT-11

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Abstract *Purpose*: It has been reported that a significant portion of the lactone form of 7-ethyl-10-(4-[1-piperidino]-1-piperidino)-carbonyloxy-camptothecin (CPT-11) is excreted into the gastrointestinal lumen via the intestinal membrane and that carboxylesterase activity, which converts CPT-11 to SN-38, was detected in the intestine. It is possible that a reduction in the excretion of CPT-11 lactone into the gastrointestinal lumen induces the gastrointestinal toxicity. The purpose of this study was to investigate the characteristics of transporter(s) that contribute to the jejunal efflux of the lactone form of CPT-11. *Methods*: The serosal-to-mucosal permeation rate of CPT-11 lactone was investigated in everted sac studies. *Results*: The secretory transport required metabolic energy and was diminished by sulfobromophthalein (BSP) and 1-naphthol, inhibitors of the ME3277 transport system. However, inhibitors of breast cancer resistance protein (Bcrp), multidrug resistance-associated protein 2 (Mrp2) and *P*-glycoprotein (*P*-gp) did not affect the secretion of CPT-11 lactone. *Conclusions*: The results suggest that a specific transport system, which is identical to the ME3277 transport system, plays a major role in the secretion of CPT-11 lactone.

Keywords Irinotecan · Diarrhea · Jejunum · Transporter

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Introduction

Irinotecan hydrochloride, 7-ethyl-10-(4-[1-piperidino]-1-piperidino)-carbonyloxy-camptothecin (CPT-11), is a synthetic derivative of the plant alkaloid camptothecin, which has demonstrated pronounced antitumor activity [11]. However, the clinical use of CPT-11 has been associated with an unexpected and significant incidence of diarrhea, and diarrhea is now recognized as a dose-limiting toxicity of CPT-11 [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.

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) [19]. Most of SN-38 undergoes subsequent conjugation by glucuronyltransferase in the liver to SN-38-glucuronide (SN-38-Glu) [5]. SN-38-Glu is excreted into the bile with the other major components, CPT-11 and SN-38 [5]. Several proposed mechanisms of this diarrhea involve biliary excretion of CPT-11 and/or its metabolites [10, 17]. 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 [22].

In clinical studies, CPT-11 was administered as a lactone form because of the antitumor activities of the lactone form of CPT-11 and its derivatives are stronger than those of the carboxylate form [24]. Recently, Arimori et al. [3, 4] reported that significant portions 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 to rats and that the amount of intestinal excretion of the lactone form of CPT-11 is considerably

greater than those of other metabolites. The amounts of cumulate biliary excretion and intestinal excretion of CPT-11 were 4.4 and 11% of the dose in the 4 h after dosing with the lactone form of CPT-11, respectively. They also suggested that the gastrointestinal impairment induced by CPT-11 might also occur as a consequence of 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.

It has been reported that the existence and state of the lactone ring of camptothecin analogs, including SN-38, are important for its antitumor activity [21, 24]. Moreover, the level of carboxylesterase activity in the generation of SN-38 from CPT-11 has been reported to be highest in the upper small intestine, followed by the lower small intestine, cecum and colon [22]. However, little is known about the transport mechanisms or transporters that contribute to the intestinal secretion of CPT-11 lactone. Thus, we focused on jejunal permeation of the lactone form of CPT-11. The aim of this study was to characterize the efflux transport system for CPT-11 lactone. The transport of CPT-11 lactone was investigated in everted sac studies.

Materials and methods

Chemicals

All chemicals and reagents used were of analytical grade. CPT-11 was kindly donated by Daiichi Pharmaceutical (Tokyo, Japan). Camptothecin, probenecid, mitoxantrone sulfobromophthalein (BSP), taurocholate and 1-naphthol were obtained from SIGMA (St. Louis, MO, USA). Verapamil was purchased from Wako Pure Chemical (Osaka, Japan). For preparation of the lactone form of CPT-11, CPT-11 was dissolved in 50 mM potassium phosphate (pH 3.0) and the sample was left overnight [8]. The conversion of the carboxylate form of CPT-11 to the lactone form was virtually complete (>99%), as determined by HPLC. Camptothecin was used as an internal standard.

Animals

Male Wistar rats, aged 6–7 weeks (300–350 g in weight), were obtained from NRC Haruna (Gunma, Japan). The housing conditions were described previously [12]. The experiment 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 [12]. Since the hydrolysis reaction of CPT-11 lactone is very fast at pH 7.4, all experiments were performed at pH 6.0 [2]. The medium used for all experiments was Tyrode's buffer (137 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 12 mM $NaHCO_3$, 0.4 mM NaH_2PO_4, and 6 mM D-glucose, pH 6.0). For everted sac studies, the jejunum was excised from the rat under anesthesia and rinsed in ice-cold saline. The intestinal segments were slid onto a glass rod and the epithelial surface was exposed. After washing the epithelial surface with ice-cold saline, 5-cm-long everted segments of intestine were isolated. These everted segments were each ligated at one end. Then 100 μl of an experimental solution containing a substrate (100 μM CPT-11 lactone) was injected into each segment, and each segment was ligated at the other end. The sac was immersed into 10 ml of drug-free buffer. The buffer was prewarmed at 37°C and preoxygenated with O_2/CO_2 (95:5) mixture gas. Under bubbling with mixture gas, the amount of the substrate transported from the serosal to mucosal surfaces across the intestine was measured by sampling the mucosal buffer periodically for 60 min. All samples were analyzed by HPLC as described below. In the energy-dependency studies, the buffer was preincubated at 37°C for 30 min in the presence of 10 mM sodium fluoride and 10 mM sodium azide.

Analytical procedures

An HPLC system equipped with a fluorescence detector was used to determine CPT-11 as described previously [13, 14]. The column was a C8 column (250×4.5 mm, 5 μm; GL Sciences). A mobile phase consisting of [50 mM monobasic potassium phosphate (pH 2.5), 7 mM tetrabutylammonium bromide]:acetonitrile (70:30, v/v) was used. 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.

Statistical analysis

Analysis of variance (ANOVA) and Student's *t* test were used for the statistical analysis, and a value of $P < 0.05$ was considered significant.

Results

Energy dependence of permeability of the lactone form of CPT-11 across rat jejunal tissue

It has been reported that intestinal exsorption of SN-38 was inhibited by cyclosporin A, a modulator of *P*-glycoprotein (*P*-gp/Abcb1), and multidrug resistance-associated protein 2 (Mrp2/Abcc2) [3]. In the first part of this study, we investigated whether CPT-11 lactone transport

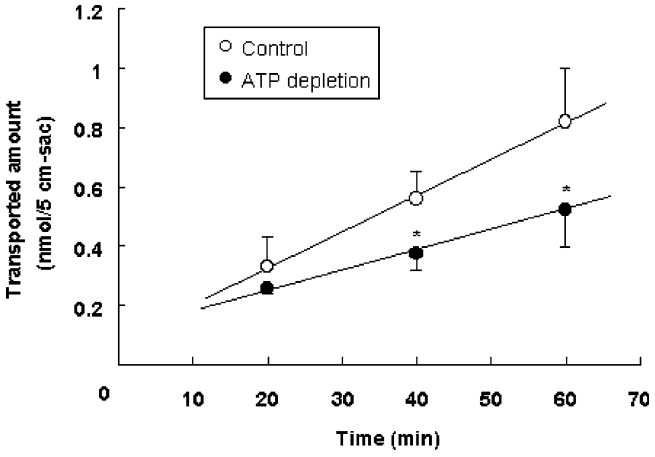

Fig. 1 Energy dependence of the serosal-to-mucosal permeation of CPT-11 lactone across the everted jejunum. The concentration of CPT-11 lactone was 100 μM. The experimental solution was adjusted to pH 6.0, and the temperature was maintained at 37°C. Each point represents the mean with SD of four separate everted sac preparations from four different rats. $*P < 0.05$, versus control

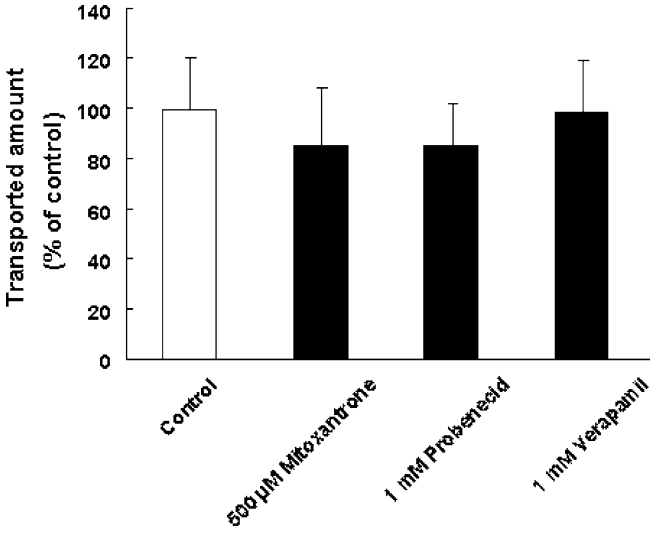

Fig. 2 Effects of ABC transporter inhibitors on the serosal-to-mucosal permeation of CPT-11 lactone across the everted jejunum. The concentration of CPT-11 lactone was 100 μM. Results were obtained at the end of a 60-min experiment. Each value represents the mean with SD of four separate everted sac preparations from four different rats. The control value for the permeation of CPT-11 lactone was 1.04 ± 0.20 nmol/5-cm sac

requires metabolic energy. The effect of metabolic inhibitors on serosal-to-mucosal permeability of the lactone form of CPT-11 was investigated (Fig. 1). The serosal-to-mucosal permeation of CPT-11 lactone was significantly decreased in the presence of metabolic inhibitors.

Effects of various compounds on permeation of CPT-11 lactone on the everted jejunum

To clarify the characteristics of transporters responsible for the secretory transport of CPT-11 lactone, the inhibitory effects of some compounds known to be inhibitors of a number of active transport systems on transport of the lactone form of CPT-11 in the jejunum were studied. The effect of mitoxantrone, an inhibitor of breast cancer resistance protein (Bcrp/Abcgz), on serosal-to-mucosal permeability of the lactone form of CPT-11 was examined [9]. As shown in Fig. 2, no significant difference was observed in the presence of mitoxantrone. Probenecid and verapamil have been reported to be typical inhibitors of Mrp2 and *P*-gp, respectively [6, 20]. They had no effect on the secretory permeation of CPT-11 lactone. It has been reported that BSP and 1-naphthol inhibit an intestinal organic anion transport system that is distinct from either *P*-gp or Mrp2 [18]. The effects of BSP and 1-naphthol on serosal-to-mucosal permeability of the lactone form of CPT-11 were examined. As shown in Fig. 3, both compounds significantly reduced serosal-to-mucosal permeation of the lactone form of CPT-11. It has been reported that Oatp1a5 (Slco1a5, previously called Oatp3, Slc21a7) is expressed in the apical membrane of the epithelium of the jejunum and that BSP is a substrate for Oatp1a5 [7, 23]. In addition to BSP, taurocholate has been reported to be a substrate for Oatp1a5 [7, 23]. In order to investigate the contribution of Oatp1a5 to the intestinal secretion of CPT-11 lactone, the effect of taurocholate on the permeation of CPT-11 lactone was examined. Taurocholate did not inhibit the secretory permeation of CPT-11 (Fig. 3).

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 [5, 22]. The antitumor activities of the lactone form of SN-38 have been shown to be stronger than those of the carboxylate form [21, 24]. It has been reported that a significant portion of the lactone form of CPT-11 was excreted into the gastrointestinal lumen via the intestinal membrane and that carboxylesterase activity, which converts CPT-11 to SN-38, was detected in the intestine [3, 4, 22]. Interestingly, relatively large amounts of SN-38 were detected in the intestinal tissues of rats and humans [16]. Thus, it is possible that reduction in the excretion of CPT-11 lactone into the gastrointestinal lumen induces the gastrointestinal toxicity. However, little is known about the characteristics of the intestinal CPT-11 transport system.

Since carboxylesterase activity gradually decreases from the jejunum to the colon, we focused on jejunal secretion of the lactone form of CPT-11 [22]. In the first part of this study, jejunal secretion of the lactone form of CPT-11 was found to require metabolic energy. Next,

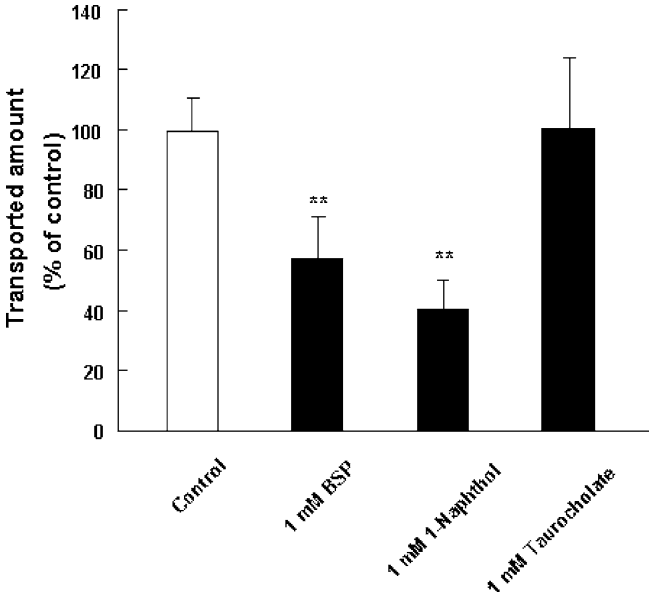

Fig. 3 Effects of BSP, 1-naphthol and taurocholate on the serosal-to-mucosal permeation of CPT-11 lactone across the everted jejunum. The concentration of CPT-11 lactone was 100 μM. Results were obtained at the end of a 60-min experiment. Each value represents the mean with SD of four separate everted sac preparations from four different rats. The control value for the permeation of CPT-11 lactone was 1.05 ± 0.11 nmol/5-cm sac. $^{**}P < 0.01$, versus control

we investigated the transport properties of jejunal CPT-11 lactone secretion. Inhibition studies were carried out using representative substrates and inhibitors of identified transporters. It has been reported that several ATP binding cassette (ABC) transporters such as Bcrp, Mrp2 and *P*-gp are present in the brush-border membrane of the intestine and that these ATP-dependent efflux transporters mediate the secretion of various compounds into the lumen [15]. Thus, the contributions of these transporters to jejunal secretion of the lactone form of CPT-11 were examined. No significant difference was observed in the presence of these transporter inhibitors. These findings suggest that Bcrp, Mrp2 and *P*-gp are not involved in the jejunal secretion of CPT-11 lactone.

Okudaira et al. [18] reported that ME3277, a glycoprotein IIB/IIIa receptor antagonist, is preferentially transported in the serosal-to-mucosal direction across the rat small intestine in the presence of glucose and that the efflux transport of ME3277 was inhibited by BSP and 1-naphthol. We focused on this transport system and examined the effects of BSP and 1-naphthol on secretion of the lactone form of CPT-11. Both of them significantly reduced the permeation of CPT-11 lactone, suggesting that the ME3277 transport system plays a major role in the secretion of CPT-11 lactone. Moreover, it was shown that probenecid and verapamil did not inhibit the efflux transport of ME3277 [18]. Our hypothesis was further supported by these findings.

In addition to the ME3277 transport system, BSP is also a substrate of Oatp1a5, which is expressed in the apical membrane of the epithelium of the jejunum [7, 23]. Thus, we investigated the contribution of Oatp1a5 to the jejunal secretion of CPT-11 lactone using taurocholate, a typical substrate for Oatp1a5 [7, 23]. Taurocholate did not affect permeation of the lactone form of CPT-11. This finding indicates that CPT-11 lactone efflux is not mediated by Oatp1a5.

In conclusion, our results suggest that a specific transport system, which is identical to the ME3277 transport system, plays a major role in the secretion of CPT-11 lactone.

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