Cancer Chemother Pharmacol (1998) 42 (Suppl): S44–S49

ORIGINAL ARTICLE

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Multiplicity of biliary excretion mechanisms for the camptothecin derivative irinotecan (CPT-11), its metabolite SN-38, and its glucuronide: role of canalicular multispecific organic anion transporter and P-glycoprotein

Abstract A frequent dose-limiting effect of irinotecan (CPT-11) is its gastrointestinal toxicity (diarrhea), which is thought to be related to biliary excretion of CPT-11 and its metabolites. Accordingly, we have investigated the mechanism of biliary excretion of these compounds. In vivo pharmacokinetic studies revealed that the biliary excretion of the four anionic forms of CPT-11 and its metabolites was reduced in Eisai hyperbilirubinemic rats, which carry a mutation of the hepatic canalicular multispecific organic anion transporter (cMOAT) gene. The protein encoded by this gene is expressed on the bile canalicular membrane and is responsible for the transport of organic anions into bile. Detailed analysis using isolated liver bile canalicular membrane vesicles to identify transport systems showed that cMOAT is responsible for biliary excretion of the low-affinity component of the carboxylate form of CPT-11 and the high-affinity component of both the lactone and carboxylate forms of SN-38 glucuronide. The carboxylate form of SN-38 is transported by cMOAT alone. Transport of the high-affinity component of CPT-11 was inhibited by verapamil and PSC-833, but their effect on the transport of its low-affinity component was minimal. In addition, ATP dependence in the uptake of CPT-11 by membrane vesicles obtained from a P-glycoprotein (P-gp)-overexpressing cell line was observed. Thus P-gp may be responsible for transport of the high-affinity component of the carboxylate form of CPT-11.

Key words Irinotecan · Biliary excretion · P-glycoprotein · cMOAT

Work presented at the 13th Bristol-Myers Squibb Nagoya International Cancer Treatment Symposium, "Strategic Cross Talk between Major Oncology Groups/Clinical Pharmacology in Cancer Chemotherapy," 17–18 October 1997, Nagoya, Japan

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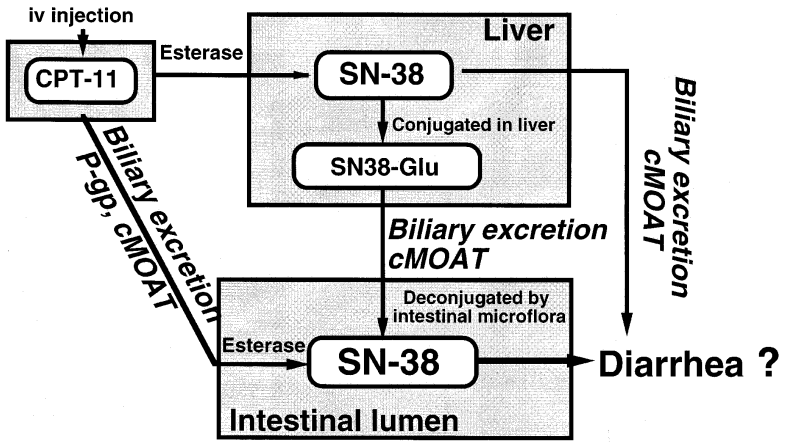
Introduction

Irinotecan (CPT-11; 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin) is a water-soluble analogue of camptothecin which was discovered in an attempt to identify derivatives with greater water solubility and antitumor activity than CPT [9]. CPT-11 is a prodrug that undergoes deesterification in vivo to yield SN-38, an active metabolite.

The major toxic effects of CPT-11 are myelosuppression and gastrointestinal toxicity, particularly unpredictable severe diarrhea [7]. This gastrointestinal toxicity, the mechanism(s) for which are currently unknown, shows wide interpatient variability [22, 23]. One postulated mechanism for the toxicity of CPT-11 is related to the biliary excretion of its metabolites (Fig. 1). After administration of CPT-11 and its subsequent deesterification to form the active metabolite SN-38, SN-38 is further conjugated to form SN-38 glucuronide (SN38-Glu) in the liver. SN38-Glu is mainly excreted via the bile duct; deconjugation by intestinal microflora to regenerate SN-38 may cause diarrhea [14]. One approach that makes use of this hypothesis to try to decrease the toxicity of CPT-11 is to reduce the gastrointestinal SN-38 concentration by inhibiting the activity of β-glucuronidase in the intestinal microflora. It has been reported that baicalin, an inhibitor of β-glucuronidase, can ameliorate CPT-11-induced diarrhea in rats [19, 24]. However, the mechanism of the biliary excretion of CPT-11 and its metabolites has not yet been identified.

CPT-11 and its metabolites have an α-hydroxy-δ-lactone ring which undergoes reversible hydrolysis at a rate which depends on many factors including pH, ionic strength, and protein concentration (Fig. 2), producing carboxylate and lactone forms of these 3 compounds. Anionic charges are present on the carboxylate forms of CPT-11 and SN-38, and on the carboxylate and lactone forms of SN38-Glu.

In our study the mechanism of biliary excretion of both the carboxylate and lactone forms of CPT-11 and its metabolites was investigated in rats [3]. Eisai hyperbilirubinemic rats (EHBRs), derived from Sprague-Dawley



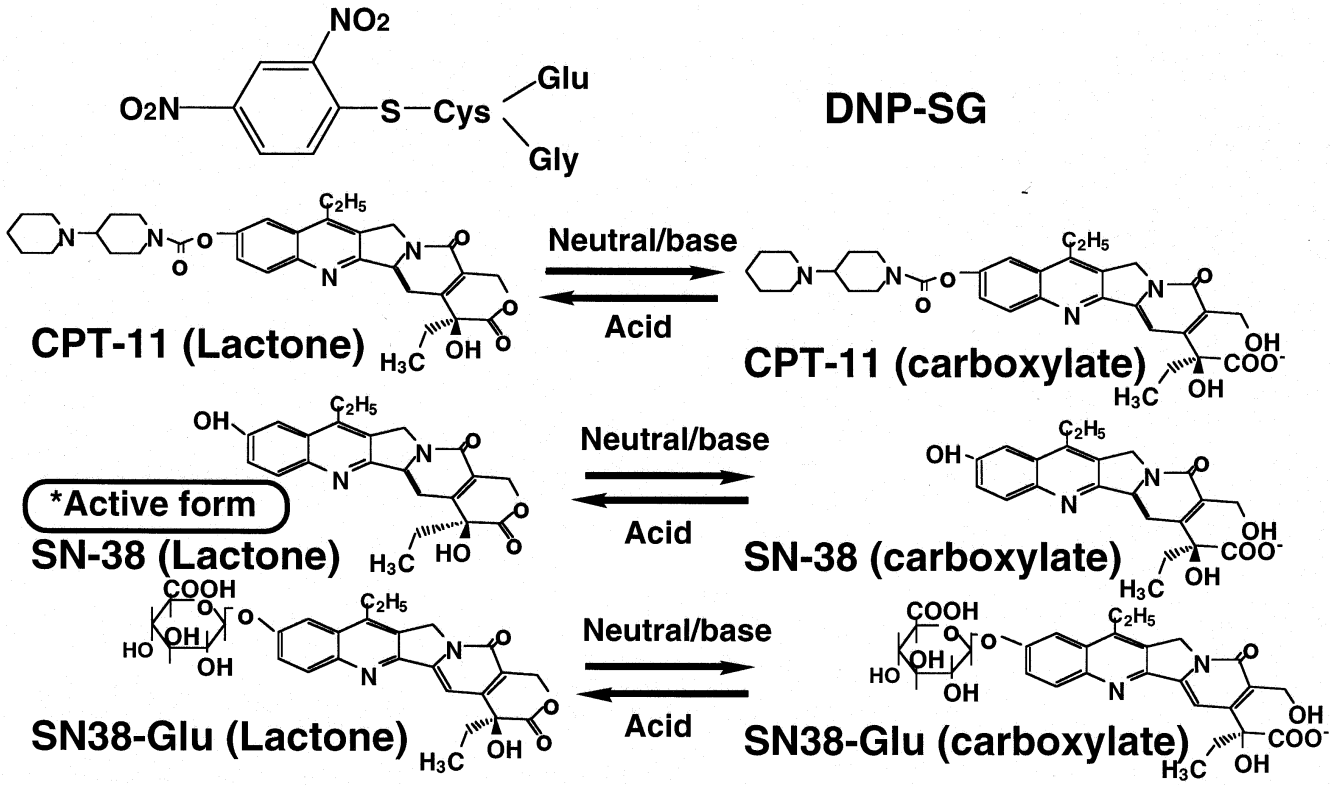


Fig. 1 Hypothesis for the mechanism of toxicity caused by intravenous administration of CPT-11

Fig. 2 Chemical structures of DNP-SG and the lactone and carboxylate forms of CPT-11 and its metabolites. Reproduced, with permission, from Chu X et al [3]

(SD) rats, were used for this purpose; EHBRs carry a canalicular multispecific organic anion transporter (cMOAT) mutation [6, 25]. The biliary excretion profiles of CPT-11 and its metabolites in SD rats and EHBRs were compared [3]. The isolated bile canalicular membrane vesicles (CMVs) from SD rats and EHBRs were also used to study the transport of CPT-11 and its metabolites [3, 4]. These studies revealed that multiple primary active transport systems are involved in the biliary excretion of CPT-11 and its metabolites. Further studies using human CMVs suggest that the primary active transport system is also involved in biliary excretion of these compounds in humans.

Biliary excretion profiles in rats in vivo [3]

After intravenous administration of the lactone form of CPT-11 (10, 25, or 40 mg/kg) to rats, both plasma concentration and biliary excretion time-profiles for CPT-11 and its metabolites were determined [3]. Additionally, the liver was excised from these rats 8 h after CPT-11 administration and the concentration of these compounds was determined to estimate directly the efficiency of transport across the bile canalicular membrane in vivo. The biliary excretion clearance ($CL_{bile,h}$) was defined as the ratio of the biliary

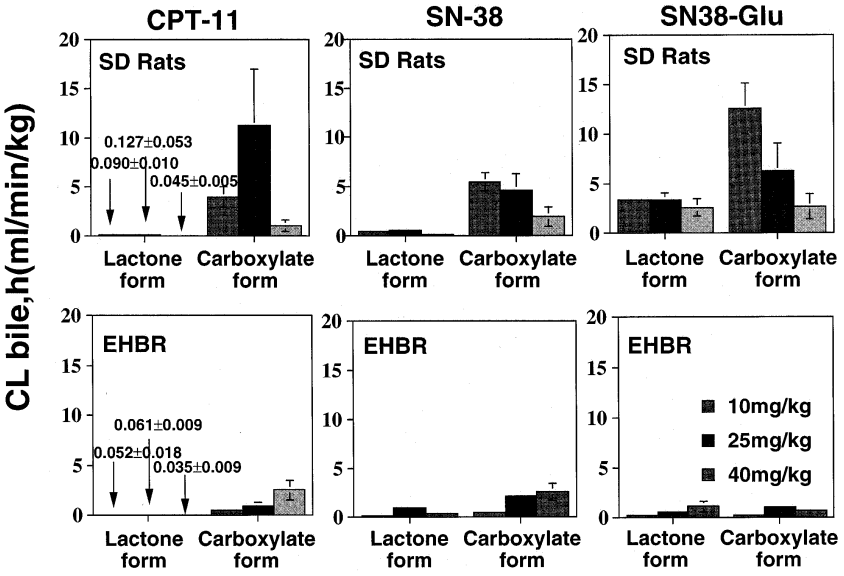


Fig. 3 Comparison of the biliary excretion clearance of CPT-11, SN-38, and SN38-Glu in SD rats and EHBRs. Reproduced, with permission, from Chu X et al [3]

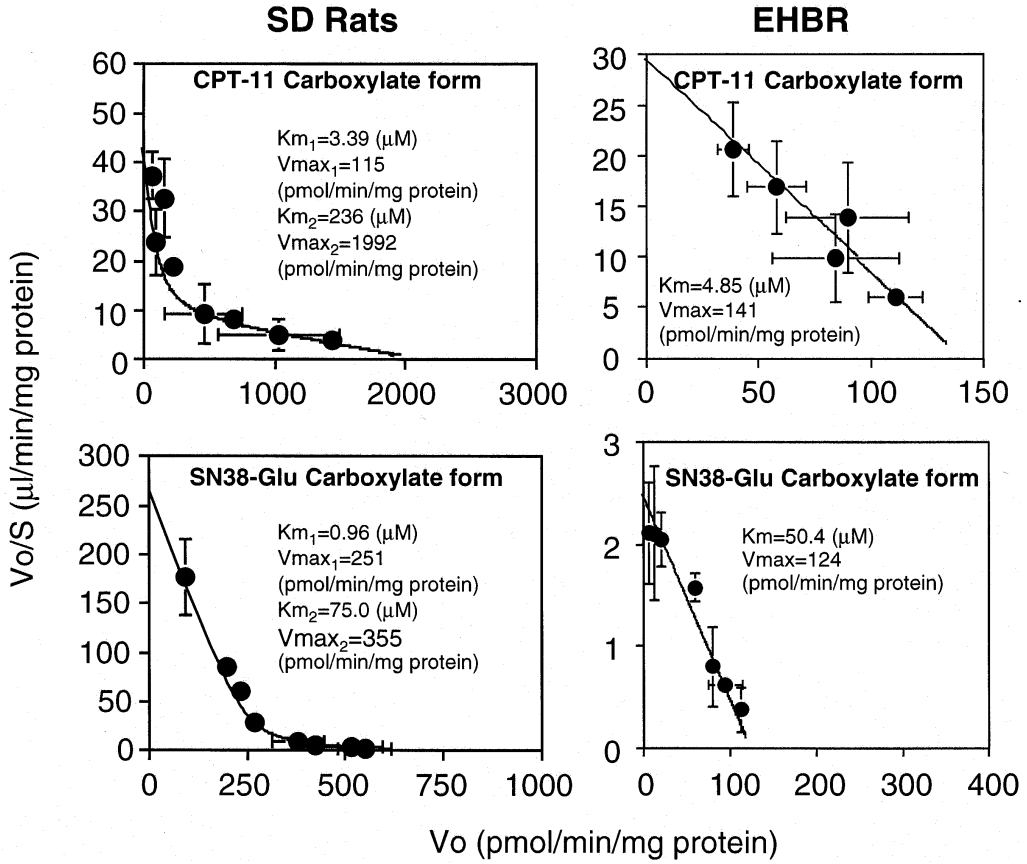


Fig. 4 Eadie-Hofstee plots for ATP-dependent uptake of the carboxylate forms of CPT-11 and SN38-Glu by SD rat and EHBR CMVs. Reproduced, with permission, from Chu X et al [4]

excretion rate ($V_{bile(8h)}$) to the liver concentration ($X_{liver(8h)}$). $V_{bile(8h)}$ was estimated using the equation:

$$V_{bile(8h)} = X_{bile(6-8h)} / 120 \text{ min}$$

where $X_{bile(6-8h)}$ is the amount of drug excreted into the bile duct from 6 to 8 h after CPT-11 administration.

In SD rats, the $CL_{bile,h}$ for anionic compounds such as the carboxylate forms of CPT-11, SN-38, and SN38-Glu and the lactone form of SN38-Glu was greater than that for the lactone forms of CPT-11 and SN-38 (Fig. 3) [3]. Thus biliary excretion across the bile canalicular membrane of anionic compounds is more efficient than that of ionic

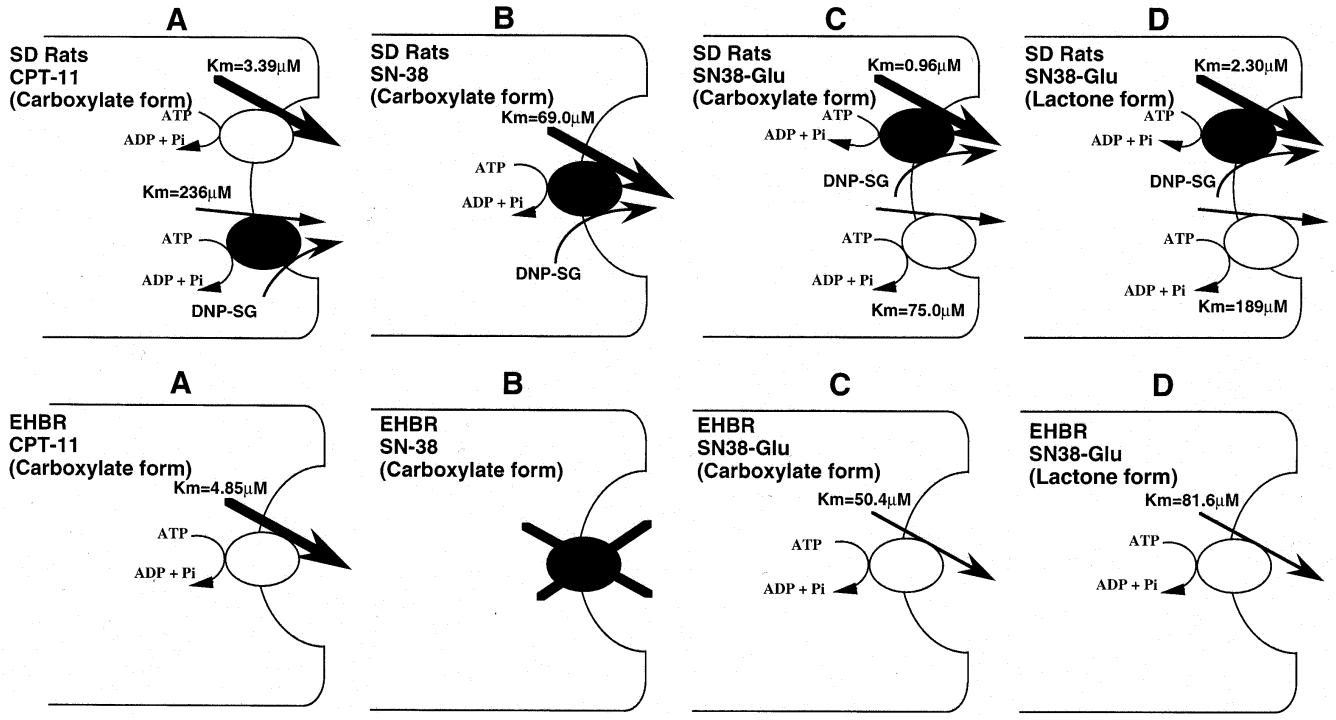


Fig. 5 Primary active transport systems for CPT-11 and its metabolites in the bile canalicular membrane of rats. Reproduced, with permission, from Chu X et al [4]

compounds. In addition, the $CL_{bile,h}$ for anionic compounds in EHBRs was smaller than that in SD rats (Fig. 3) [3]. Thus cMOAT is responsible for the biliary excretion of these four compounds in rats.

Primary active transport of CPT-11 and its metabolites in CMVs

Involvement of cMOAT [3, 4]

Kinetic analysis of the transport of the 4 anionic compounds was performed using isolated CMVs from both SD rats and EHBRs. The uptake of these compounds by CMVs from SD rats showed ATP dependence [3], and for the carboxylate forms of CPT-11 and SN38-Glu showed biphasic saturation with high- and low-affinity components (Fig. 4) [4]. Similar kinetic profiles were also observed for the lactone form of SN38-Glu, while ATP-dependent uptake of the carboxylate form of SN-38 showed a single saturable component. All 4 anionic compounds inhibited the ATP-dependent uptake of DNP-SG, a representative substrate for cMOAT in rats; the inhibition constants (Ki) are shown in Table 1 [3]. ATP-dependent uptake of the anionic compounds by EHBR CMVs was lower, suggesting the involvement of cMOAT in active transport across the bile canalicular membrane. Minor, but saturable and significant ATP-dependent uptake of the carboxylate form of CPT-11 and SN38-Glu, and the lactone form of SN38-Glu by EHBR CMVs was observed. The kinetic parameters obtained for the uptake by SD rat and EHBR CMVs are listed in Table 1 [4].

cMOAT is involved in the transport of the low-affinity component of the carboxylate form of CPT-11 because 1) no low-affinity component with a Km of 200–300 μM was found in EHBR CMVs and 2) the Km for the low-affinity component was comparable with the Ki for ATP-dependent DNP-SG uptake (Table 1, Fig. 5). In contrast, cMOAT is involved in the transport the high-affinity component of both carboxylate and lactone forms of SN38-Glu because 1) the high-affinity component with a Km of 1–3 μM was not found in EHBR CMVs and 2) the Km for the high-affinity component was comparable with the Ki (Table 1, Fig. 5). This conclusion is supported by the finding that inhibition

Table 1 Comparison of Km for the uptake of CPT-11 and its metabolites and Ki for DNP-SG uptake [3, 4]

Compound	Rat strain	Km_1 (μM)	Km_2 (μM)	Ki (μM)
CPT-11	SD	3.39 ± 3.07	236 ± 112	96.6 ± 8.4
carboxylate form	EHBR	4.85 ± 0.86		
SN38-Glu	SD	0.96 ± 0.08	75.0 ± 15.0	1.03 ± 0.05
carboxylate form	EHBR		50.4 ± 3.4	
SN38-Glu	SD	2.30 ± 0.25	189 ± 44	1.62 ± 0.05
lactone form	EHBR		81.6 ± 15.8	

of the ATP-dependent uptake of the carboxylate form of CPT-11 by DNP-SG was more marked at a higher (250 μM) CPT-11 concentration than at a lower (5 μM) concentration, while such inhibition of the carboxylate form of SN38-Glu was more marked at a lower (5 μM) SN38-Glu concentration than at a higher (250 μM) concentration [4]. Thus, multiple primary active transport systems are suggested to exist for the biliary excretion of the four anionic compounds in rats.

Recently, the cMOAT gene was identified and cloned [2, 12, 21]; in addition, cMOAT expression has been found to be defective in several strains of hyperbilirubinemic rats, including the TR^- and EHBR strains, due to cMOAT gene mutations, the sites of which have been investigated [12, 21]. However, we have reported that a primary active transport system for organic anions is still present on the bile canalicular membrane of EHBRs [20, 25, 26]; Niinuma et al found that there was ATP-dependent uptake of the glucuronide conjugate of E3040 by both SD rat and EHBR CMVs [20]. Attempts are currently being made to determine how many transport proteins for organic anions exist [25, 26]. We have identified a gene fragment amplified from the ATP-binding cassette (ABC) region using the polymerase chain reaction which hybridizes to poly $(A)^+$RNA from the liver of EHBRs [11]. This may be a transporter which recognizes the glucuronide conjugate of E3040 and exists in EHBRs.

Involvement of P-glycoprotein

ATP-dependent uptake of the carboxylate form of CPT-11 (5 μM) by SD rat CMVs was inhibited by verapamil, cyclosporin A, and PSC-833, but their effect on the uptake of CPT-11 250 μM was relatively small and comparable to their effect on the uptake of DNP-SG. In contrast, the uptake of CPT-11 250 μM was inhibited by DNP-SG while that at 5 μM was not inhibited to the same extent. These results suggest that the high-affinity site for the carboxylate form of CPT-11 involves P-glycoprotein. Gupta et al. [8] reported that photoaffinity-labeling of P-gp using a photoaffinity analogue of verapamil is inhibited by CPT-11, SN-38, and SN38-Glu, suggesting that these compounds have affinity for P-gp. Additionally, Gupta and colleagues found that pretreatment with cyclosporin A, a well-known substrate and inhibitor of P-gp, results in an increase in the area under the plasma concentration-time curve of CPT-11 and its metabolites in rats. These findings are compatible with our suggestion that P-gp is also involved in the biliary excretion of CPT-11 in rats. However, cyclosporin A could also inhibit the primary active transport mediated by cMOAT or a bile acid transporter [1]. P-gp-mediated primary active transport of CPT-11 is also supported by our finding that the uptake of CPT-11 by membrane vesicles obtained from human cell lines overexpressing P-gp is ATP dependent while uptake by membrane vesicles from parent cell lines is not.

Primary active transport of CPT-11 and its metabolites in humans

We found that the uptake of the 4 anionic CPT-11-related compounds by CMVs obtained from humans is also ATP dependent. This suggests that there is a primary active transport system in the bile canalicular membrane in human liver. The uptake of CPT-11-related compounds by human CMVs varies widely between CMV samples, which may imply wide interindividual differences in the biliary excretion of CPT-11 and its metabolites.

Relevance to multidrug resistance in cancer cells

Cole et al. [5] found that the multidrug resistance-associated protein (MRP), a member of the ABC transmembrane transport superfamily which has a common ABC region [10, 26], is overexpressed in a multidrug-resistant human lung cancer cell line. A recent investigation demonstrated that MRP also transports the endogenous glutathione conjugate leukotriene C_4 (LTC_4), the endogenous glucuronide conjugate estradiol 17-(β-D-glucuronide), and other organic anions in an ATP-dependent manner [13, 15–17], with substrate specificity similar to that of cMOAT. These observations imply that it is possible that MRP or MRP-related transporters might be involved in the efflux transport of the 4 anionic CPT-11 related compounds and methotrexate [18], which has also been demonstrated to be excreted into bile by cMOAT in rats, from cancer cells overexpressing MRP. We found that the carboxylate forms of SN-38 and SN38-Glu, and the lactone form of SN38-Glu are taken up by membrane vesicles formed from human cancer cell lines overexpressing MRP in an ATP-dependent manner. Thus, these three compounds are substrates for MRP in humans.

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