Active Efflux of CPT-11 and Its Metabolites in Human KB-Derived Cell Lines

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ABSTRACT

To investigate the possible involvement of P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP), and/or other glutathione S-conjugate export pump (GS-X pump) family members on the active efflux of irinotecan [(7-ethyl-10-[4-(1-piperidino)-1-piperidino)-1-piperidino]carboxyloxy camptothecin (CPT-11)] and its metabolites, as well as their contribution to the acquisition of resistance, we studied the uptake of CPT-11, its active metabolite SN-38, and glucuronide conjugate (SN38-Glu) using membrane vesicles from human epidermoid KB-3-1-derived cell lines. These lines included KB-C2, C-A500, and KCP-4, which overexpress P-gp, MRP, and the unidentifiable GS-X pump, respectively. The carboxylate form of SN-38 exhibited significant ATP-dependent transport, with a Michaelis constant of 17 μM, into membrane vesicles from C-A500 but not from other cell lines. Among these KB-derived cells, significant ATP-dependent uptake of the carboxylate form of CPT-11 was only observed in KB-C2 vesicles. In addition, the uptake of the lactone and carboxylate forms of SN38-Glu into membrane vesicles from C-A500 and KB-C2, but not KCP-4, was ATP dependent, although the transport activity in C-A500 was much higher than that in KB-C2. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay revealed that the resistance of KB-C2 to CPT-11 and SN-38, compared with that of KB-3-1, was 6.3- and 6.8-fold, respectively; the corresponding figures for C-A500 were 12- and 27-fold, respectively, whereas those for KCP-4 were 2.3- and 20-fold, respectively. These results suggest that MRP and P-gp are involved in the active efflux of SN-38 and CPT-11, respectively, from human KB-derived cells. In addition, a difference in substrate specificity among GS-X pump members was demonstrated.

Multidrug resistance, one of the major obstacles encountered in cancer chemotherapy, is often acquired by overexpression of ATP-binding cassette transmembrane transporters such as P-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP) (Gottesman et al., 1996; Lautier et al., 1996; Loe et al., 1996). Although the overexpression of P-gp and MRP provides almost the same spectrum of resistance to antitumor drugs, the substrate specificity of these transporters determined in isolated plasma membrane vesicles differ markedly. In general, P-gp accepts amphibathic cationic or neutral compounds as a substrate, whereas MRP acts as a glutathione S-conjugate export pump (GS-X pump) (Ishikawa and Akimura, 1996; Lautier et al., 1996; Loe et al., 1996). However, the ATP-dependent glutathione S-conjugate export pump, cMOAT, canalicular multispecific organic anion transporter; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

ABBREVIATIONS: CMV, canalicular membrane vesicles; CPT, camptothecin; CPT-11, (7-ethyl-10-[4-(1-piperidino)-1-piperidino)-1-piperidino]carboxyloxy camptothecin; SN38-Glu, SN38-glucuronide; P-gp, P-glycoprotein; MRP, multidrug resistance-associated protein; GS-X pump, ATP-dependent glutathione S-conjugate export pump; cMOAT, canalicular multispecific organic anion transporter; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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tion of cloned cMOAT (Buchler et al., 1996; Paulusma et al., 1996; Taniguchi et al., 1996; Ito et al., 1997; 1998; Borst et al., 1997; Madon et al., 1997; Evers et al., 1998; Koike et al., 1997; van Aubel et al., 1998). Moreover, we found that several antitumor drugs are extruded via cMOAT, including methotrexate, irinotecan (7-ethyl-10-(4-(1-piperidino)-1-piperidino)-carbonylcamptothecin) (CPT-11) and its metabolites (Chu et al., 1997a,b; Masuda et al., 1997).

CPT-11 is a derivative of camptothecin (CPT) with substantial anticancer activity in a broad spectrum of human tumor cells (Slichenmyer et al., 1993). CPT-11 is a prodrug that can be converted by carboxyl esterase to its active metabolite, SN-38, which is able to inhibit topoisomerase I (Slichenmyer et al., 1993). The α-hydroxy-δ-lactone ring in CPT-11 and SN-38 is in equilibrium with its carboxylate form, and the equilibrium reaction favors the production of the carboxylate form at physiological pH and the lactone form at pH values below physiological (Fassberg and Stella, 1992). We found that cMOAT is responsible for the biliary form, and the equilibrium reaction favors the production of CPT-11 and SN-38.

In addition, the results of the transport studies were compared with the P-gp pump (Fujii et al., 1994; Chuman et al., 1996). In addition, the role of these transporters in the acquisition of resistance. 

According to Northern blot analysis of poly(A)+ RNA using cDNA fragments of several primary active transporters (K Ueda, H Suzuki, S Akiyama, and Y Sugiyama, submitted), KB-C2 and C-A500 overexpressed P-gp and MRP, respectively. In addition to MRP, C-A500 also expressed human cMOAT. However, the expression level of cMOAT in C-A500 was much lower than that of MRP but higher than that in KB-3-1 cells. The detectable MRP3, a MRP homolog (Hiromi et al., 1998), was also found in C-A500; however, its expression was comparable with that in KB-3-1 cells. As far as KCP-4 is concerned, neither P-gp, MRP, cMOAT, nor MRP3 was overexpressed.

**Isolation of Membrane Vesicles.** Membrane vesicles were prepared from above KB-derived cells by the nitrogen cavitation method (Ito et al., 1998). Cell monolayers (105–106 cells) were washed once and scraped into phosphate-buffered saline. The cells were washed by centrifugation (4,000g, 10 min) at 4°C in phosphate-buffered saline and then in buffer A consisting of 250 mM sucrose, 0.2 mM CaCl2, and 10 mM Tris/HCl (pH 7.4) and equilibrated at 4°C under a nitrogen pressure of 900 psi for 15 min. EDTA was added to the cell lysate to give a final concentration of 1 mM. The lysed cell suspension was then diluted with 4 volumes of buffer B containing 250 mM sucrose and 10 mM Tris/HCl (pH 7.4) and centrifuged at 4°C under a nitrogen pressure of 900 psi for 15 min.

The supernatant was layered onto 35% sucrose cushion at 100,000g at 4°C. The supernatant was centrifuged for 45 min at 100,000g at 4°C. The interface was collected, diluted 5-fold with buffer B, and then centrifuged for 45 min at 100,000g.

**Materials and Methods**

**Materials.** CPT-11, SN-38, and SN38-Glu were kindly provided by Daiichi Pharmaceutical Co. Ltd. (Tokyo, Japan) and Yakult Honsha Co. Ltd. (Tokyo, Japan). The lactone and carboxylate forms of CPT-11, SN-38, and SN38-Glu were produced by dissolving the compounds in 50 mM phosphate buffer at pH 3.0 or 9.0, respectively, and KCP-4, a cisplatin-resistant cell line. 

**Cell Culture.** Human epidermoid carcinoma KB-3-1 cells, multidrug-resistant KB-C2 (Akiyama et al., 1985), cefaparantine (1 μg/ml), doxorubicin (0.5 μg/ml), and mezezin (0.065 μg/ml) for C-A500 (Sumizawa et al., 1994), and cisplatin (7 μg/ml) for KCP-4 (Fujii et al., 1994; Chuman et al., 1996).

**Uptake by Membrane Vesicles.** The uptake of the carboxylate and lactone forms of CPT-11 and its metabolites was studied as described previously (Chu et al., 1997a,b). The uptake study was performed at 37°C in medium (20 μl) containing 250 mM sucrose, 10 mM Tris/HCl (pH 7.4), 10 mM MgCl2, 5 mM ATP, 10 mM creatine phosphate, and 100 μg/ml creatine phosphokinase. For the control experiments, ATP was replaced by AMP. The final concentration of membrane vesicles was adjusted to 0.25 mg/ml. In the present study, we examined the uptake of ligands into membrane vesicles at 2 min because our previous studies have suggested that the uptake of CPT-11 and its metabolites (anionic form) was significantly stimulated by ATP and shows linearity for at least 2 min in canaliculair membrane vesicles (CMVs) from both humans and rats (Chu et al., 1997a, 1998). Transport was terminated by adding 1 ml ice-cold stop solution followed by immediate filtration through a 0.45-μm filter (HAWP 02500; Millipore Corporation, Bedford, MA), and subsequently washing twice with 5-ml ice-cold stop solution. The stop solution consisted of 10 mM Tris/HCl (pH 7.4), 250 mM sucrose, and 100 mM NaCl. ATP-dependent uptake was determined as the difference in uptake in the presence and absence of ATP.

Analysis of the carboxylate and lactone forms of CPT-11 and its metabolites associated with the membrane vesicles on filters and in medium was accomplished by HPLC as described previously (Chu et al., 1997a,b). Our preliminary studies indicated that the conversion
between the lactone and carboxylate forms of a series of ligands during the transport experiments was less than 5%. The limit of detection was 0.0044, 0.01, and 0.0074 pmol for SN38-Glu, SN-38, and CPT-11, respectively.

**Resistance to CPT-11 and SN-38.** The resistance of KB-3-1-derived cells to CPT-11 and SN-38 was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay performed in 96-well plates (Carmichael et al., 1987). Cells (2 \times 10^5 for KB-3-1 and KB-C-2 and 5 \times 10^5 for C-A500 and KCP-4) were seeded to each well and cultured with 50 μl of phenol red-free culture medium. After overnight incubation at 37°C in an atmosphere of 5% CO₂, CPT-11 and SN-38 were added to the cells and incubated for 4 days. Then, MTT was added to each well and incubated for 4 h. The resulting formazan was dissolved in 100 μl of dimethyl sulfoxide. Plates were placed on a plate shaker for 5 min and the absorbance at 595 nm was immediately read using a microplate reader (model 3550; Bio-Rad, Hercules, CA).

**Data Analysis.** The Eadie-Hofstee plot was used to express the concentration dependence of ATP-dependent uptake. ATP-dependent uptake was obtained by subtracting the uptake in the presence of AMP from that in the presence of ATP. Kinetic parameters for the ATP-dependent uptake were obtained by fitting using the following equation:

\[ V_u = \frac{V_{max} \times S}{(K_m + S)} \]  

(1)

where \( V_u \) is the initial uptake rate of substrate (pmol/min/mg protein), \( S \) is the substrate concentration in the medium (μM), \( K_m \) is the Michaelis constant (μM), and \( V_{max} \) is the maximum uptake rate (pmol/min/mg protein). The uptake data were fitted to eq. 1 by a nonlinear least-squares method using the MULTI program (Yamaoka et al., 1981) to obtain estimates of kinetic parameters. The input data were weighted as the reciprocal of the square of the observed uptake.

**Statistical Methods.** The results are shown as means ± S.E. for the number of determinations. Student’s t test was used to determine the significant difference between the means of two groups, with \( P < .01 \) and \( P < .05 \) as the minimum levels of significance.

**Results**

**Uptake of Carboxylate and Lactone Forms of SN-38-Glu by Membrane Vesicles from KB-Derived Cells.** Uptake of the carboxylate form of SN38-Glu (50 μM) into membrane vesicles from KB-3-1, KB-C2, C-A500, and KCP-4 cells was shown in Fig. 1A. The uptake was significantly stimulated by ATP in C-A500 and KB-C2 vesicles (Fig. 1A), although the transport activity in KB-C2 was much lower than that in C-A500 (Fig. 1A). By contrast, no significant ATP-dependence was found in KB-3-1 and KCP-4 vesicles (Fig. 1A). Similar results were obtained for the uptake of the lactone form of SN38-Glu (50 μM) (Fig. 1B), although the transport activity for the lactone form of SN38-Glu was lower than that of its corresponding carboxylate form in C-A500 vesicles (Fig. 1, A and B).

**Uptake of Carboxylate Forms of SN-38 and CPT-11 by Membrane Vesicles from KB-Derived Cells.** For the carboxylate form of SN-38 (50 μM), ATP-dependent uptake was observed only in C-A500 vesicles (Fig. 1C). No significant ATP dependence was found in KB-3-1, KB-C2, or KCP-4 vesicles (Fig. 1C). Kinetic analysis revealed that the ATP-dependent uptake of the carboxylate form of SN-38 by C-A500 vesicles consists of a single saturable component with a \( K_m \) of 17 μM and a \( V_{max} \) of 1.18 nmol/min/mg protein (Fig. 2).

The uptake of the carboxylate form of CPT-11 was also examined. Because no significant ATP-dependent uptake

![Fig. 1. Uptake of the carboxylate and lactone forms of SN38-Glu and the carboxylate form of SN-38 by membrane vesicles. Membrane vesicles from KB-3-1, KB-C2, C-A500 and KCP-4 cells (5 μg protein) were incubated with 50 μM the carboxylate form of SN38-Glu (A), the lactone form of SN38-Glu (B), and the carboxylate form of SN-38 (C) in the presence of 5 mM ATP (closed column) or AMP (open column) and ATP-regenerating system for 2 min. Data are mean ± S.E. of five different experiments. **P < .01, significantly different from that in the absence of ATP.](https://example.com/fig1.png)
was observed in membrane vesicles from all cell lines at a substrate concentration of 50 µM (Fig. 3B), the uptake study was repeated at 5 µM. At this concentration, significant ATP-dependent uptake was observed but only in KB-C2 vesicles (Fig. 3A). The uptake of the carboxylate form of CPT-11 into the membrane vesicles from C-A500 is comparable with that in KB-3-1 vesicles at both 5 and 50 µM and no ATP-dependent uptake was observed when the substrate concentration was repeated at 5 µM (Fig. 3). Although its uptake into KCP-4 vesicles in the presence or absence of ATP was slightly higher than that in KB-3-1 vesicles at 5 µM, comparable uptake was observed when the substrate concentration was 50 µM. However, no ATP-dependent uptake into KCP-4 was found at 5 or 50 µM (Fig. 3).

Resistance of KB-Derived Cells to CPT-11 and SN-38. The resistance of KB-derived cells to CPT-11 and SN-38 was determined by MTT assay. As shown in Table 1, the resistance of these cells to CPT-11 and SN-38 was increased 6.3- and 6.8-fold for KB-C2 cells compared with KB-3-1 cells, respectively, whereas the corresponding figures for C-A500 cells were 12- and 27-fold, respectively, and for KCP-4 cells 2.3- and 20-fold, respectively.

Discussion

Although the overexpression of transport proteins on tumor cells has been reported to be one of the mechanisms for the acquisition of resistance to CPT-11, a promising anticancer agent in clinical situations, no detailed mechanism has been proposed (Minato et al., 1990; Takigawa et al., 1992; Hasegawa et al., 1995). Because our previous studies suggested that CPT-11 and its metabolites are substrates for cMOAT (Chu et al., 1997a,b), cMOAT and/or related transporters might be involved in the active efflux of these drugs from tumor cells. To provide a deeper insight into the mechanism of resistance to CPT-11 acquired by the overexpression of efflux transporters and to examine the substrate specificity of P-gp and GS-X family members, uptake studies involving membrane vesicles isolated from KB-derived cell lines were performed.

For the carboxylate and lactone forms of SN38-Glu, marked ATP-dependent uptake was observed in C-A500 vesicles (Fig. 1, A and B), suggesting that the transporter, which is predominantly responsible for the efflux of this conjugated metabolite, is MRP. According to Northern blot analysis, we found that C-A500 cells also overexpressed cMOAT, although its expression level was much lower than that of MRP (K Ueda, H Suzuki, S Akiyama, and Y Sugiyama, submitted). Because our previous study with isolated rat bile CMVs also indicated that SN38-Glu is a high-affinity substrate for cMOAT (Km:s for the lactone and carboxylate forms are 2.30 and 0.96 µM, respectively) (Chu et al., 1997b), it is possible that cMOAT may also be involved in the ATP-dependent transport of SN38-Glu in C-A500. As shown in Fig. 1, A and B, the ATP-dependent uptake of the carboxylate form of SN38-Glu into C-A500 and KB-C2 vesicles is higher than that of its lactone form, which is consistent with our previous findings in rat CMVs (Chu et al., 1997b). These results can be explained if we consider the previous hypothesis that divalent anions are better substrates for cMOAT and MRP (Oude Elferink et al., 1995) and the fact that the carboxylate form of SN38-Glu has one additional anionic charge compared with its lactone form.

Significant ATP-dependent uptake of the carboxylate and lactone forms of SN38-Glu was also observed in KB-C2 vesicles where P-gp is overexpressed, although the transport activity in KB-C2 was much lower compared with that in C-A500 (Fig. 1, A and B). The suggestion that SN38-Glu is
also a substrate for P-gp is supported by the finding that some glucuronides can be substrates for P-gp. Vore et al. (1996) reported that estradiol 17-β,δ-glucuronide (E217G), a typical substrate for MRP and cMOAT, may also be transported via P-gp, based on the finding that the ATP-dependent uptake of E217G into rat CMVs was inhibited by C219, a monoclonal antibody against P-gp. In addition, we have also reported that the primary active transporter(s), which is also expressed in Eiai hyperbilirubinemic rat CMVs, is responsible for the ATP-dependent uptake of SN38-Glu in rat CMVs with low affinity (Km for the lactone and carboxylate forms were 189 and 75 μM, respectively) (Chu et al., 1997b). In contrast, no significant ATP-dependent uptake of SN38-Glu was found in KCP-4 vesicles (Fig. 1, A and B), although glutathione conjugates (LTC4 and DNP-SG) are taken up into the same vesicle preparations (Fujii et al., 1994; Chuman et al., 1996). These results, along with our recent observation that no ATP-dependent uptake of E217G is detectable in KCP-4 vesicles (K Ueda, H Suzuki, S Akiyama, and Y Sugiyama, submitted), indicate that the unidentified GS-X pump expressed on KCP-4 may exhibit a different substrate specificity from that of MRP, particularly as far as recognition of the glucuronide moiety is concerned.

As shown in Fig. 1C, ATP-dependent uptake for the carboxylate form of SN-38 was observed only in C-A500 vesicles, suggesting that MRP is involved in the active efflux of this compound. Although cMOAT was also expressed in C-A500, its expression level was considerably lower than that of MRP (K Ueda, H Suzuki, S Akiyama, and Y Sugiyama, submitted). Moreover, no ATP-dependent uptake in KB-3-1 vesicles was found in which cMOAT was also detected. By comparing the transport affinity of the carboxylate form of SN-38 in C-A500 vesicles (Km = 17 μM) with its ATP-dependent uptake in rat and human CMVs, we found that the Km of this compound was 69 and 180 μM for rat and human cMOAT, respectively (Chu et al., 1997b, 1998). This comparison suggested that the affinity of the carboxylate form of SN-38 in C-A500, which overexpresses MRP, is approximately 10-fold higher than that for cMOAT from the same species. In addition, this compound was not transported via a GS-X pump expressed on KCP-4 cells (Fig. 1C). Taking these results into consideration, we speculate that MRP is predominantly responsible for the ATP-dependent uptake of the carboxylate form of SN-38 in C-A500, whereas the contribution of cMOAT to its active efflux is minor.

The transport properties determined in membrane vesicles need to be discussed in relation to the resistance of tumor cells to antitumor drugs. The resistance of C-A500 cells to SN-38 determined by MTT assay was 27-fold higher than that of KB-3-1 cells (Table 1). Taking this together with the finding that SN-38 is a good substrate for MRP, suggests that MRP is responsible for the resistance to this drug. In contrast, the results with KB-C2 vesicles (Fig. 1C) indicate that P-gp may play only a very minor role in the resistance to SN-38. Indeed, the resistance of KB-C2 cells to SN-38 (6.8-fold higher than that of KB-3-1 cells) was much lower than that of C-A500 cells (Table 1). Our observations are further supported by the finding of Hoki et al. (1997) that the transfection of a plasmid containing cDNA for wild-type P-gp does not confer any resistance to SN-38 in NIH/3T3 cells.

Although no significant ATP-dependent uptake of the carboxylate form of SN-38 was found in KCP-4 vesicles (Fig. 1C), MTT assay revealed that KCP-4 cells also exhibited a similar resistance to SN-38 (20-fold higher than that of KB-3-1 cells) as that of C-A500 cells (Table 1). Although we cannot ignore the possibility that the lactone form of SN-38, whose antitumor activity is much more potent than its carboxylate form, is extruded via a GS-X pump expressed on KCP-4 cells, this may not be plausible because it is believed that the GS-X pump can accept only anionic compounds as substrates (Lautier et al., 1996; Loe et al., 1996). The resistance of KB-C2 and KCP-4 cells to SN-38 may be accounted for other mechanism(s), such as alterations in the expression level of topoisomerase and/or mutations in the topoisomerase gene (Slichenmyer et al., 1993; Tanizawa et al., 1993).

The involvement of P-gp in the extrusion of CPT-11 is supported by the finding that the uptake of CPT-11 into KB-C2 vesicles is stimulated by ATP at a substrate concentration of 5 μM. In addition, no ATP-dependent uptake of the carboxylate form of CPT-11 was observed in membrane vesicles from C-A500 and KCP-4, as well as KB-3-1, at both 5 and 50 μM. This suggested that active efflux of the carboxylate form of CPT-11 is not related to MRP or the GS-X pump. These results should be discussed in relation to our recent studies in which we reported that the uptake of the carboxylate form of CPT-11 into rat CMVs consists of a high (Km = 3.4 μM and Vmax = 115 pmol/min/mg protein) and a low (Km = 236 μM and Vmax = 1.99 nmol/min/mg protein) affinity component, the latter being attributed to cMOAT (Chu et al., 1997b). Our recent finding that the high-affinity component was inhibited by several drugs such as PSC-833, cyclosporin A, and verapamil (Sugiyama et al., 1998) suggests that P-gp is involved in the efflux of this compound. If we consider the transport activity under linear conditions (Vmax/Km), the contribution of P-gp to the uptake of CPT-11 by CMVs is approximately four times higher than that of cMOAT (Chu et al., 1999).

TABLE 1

<table>
<thead>
<tr>
<th>Resistance of KB-derived cell lines to CPT-11 and SN-38</th>
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<tr>
<td>Resistance of KB-derived cell lines to CPT-11 and SN-38 was determined by MTT assay. All values were given as the mean ± calculated S.D. from 1–4 different experiments of triplicate determinations. SD value of relative resistance was calculated according to law of propagation of errors, considering deviation of IC50 in KB-3-1 cells.</td>
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<tr>
<th>Cell Line</th>
<th>CPT-11 IC50 μM</th>
<th>Relative Resistance a</th>
<th>SN-38 IC50 μM</th>
<th>Relative Resistance b</th>
</tr>
</thead>
<tbody>
<tr>
<td>KB-3-1</td>
<td>27.8 ± 5.30</td>
<td>1.00 ± 0.27</td>
<td>0.52 ± 0.24</td>
<td>1.00 ± 0.65</td>
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<tr>
<td>KB-C2</td>
<td>174.0 ± 8.00</td>
<td>6.28 ± 1.23</td>
<td>3.51 ± 0.80</td>
<td>6.75 ± 3.47</td>
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<tr>
<td>C-A500</td>
<td>331.0 ± 76.00</td>
<td>11.90 ± 3.60</td>
<td>14.00 ± 3.30</td>
<td>27.00 ± 14.00</td>
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<tr>
<td>KCP-4</td>
<td>62.70 ± 14.00</td>
<td>2.26 ± 0.66</td>
<td>10.50 ± 1.80</td>
<td>20.20 ± 9.90</td>
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a IC50 was calculated with eq. (2) in text.
b Relative resistance is defined as IC50 of resistance cell lines divided by that of KB-3-1.
Akiyama S, Foji A, Hanover JA, Pastan I and Gottesman MM (1985) Isolation and SN-38 may be efficiently transported by KB-C2 vesicles (Fig. 3) is in good agreement with the fact that KB-C2 exhibited a 6-fold resistance to this compound compared with KB-3-1 cells (Table 1). It has also been reported that a bladder cancer cell line (KK47/ADM) and etoposide-resistant small-cell lung cancer cell lines (H69/VP and SBC-3/ETP), both of which overexpress P-gp, show cross-resistance to CPT-11 (Minato et al., 1990; Takigawa et al., 1997b), suggesting that CPT-11 is preferentially extruded via MRP and P-gp are involved in the active efflux of SN-38 and CPT-11, respectively, from human KB-derived cells and conferred drug resistance. In conclusion, a difference in the substrate specificity among GS-X pump members was demonstrated in that SN-38 and its glucuronide can be a substrate for MRP, but not for a GS-X pump expressed on cisplatin-resistant KCP-4 cells.

References