Improvement of the Oral Drug Absorption of Topotecan through the Inhibition of Intestinal Xenobiotic Efflux Transporter, Breast Cancer Resistance Protein (BCRP), by Excipients

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Abbreviations : BCRP (Bcrp), breast cancer resistance protein; AUC, the area under the plasma concentration-time curve; P-gp, P-glycoprotein; ABC, ATP-binding cassette; PBS, phosphate-buffered saline; HPLC, high-pressure liquid chromatography; TTBS, Tris-buffered saline with 0.05 % Tween 20; i.v., intravenous

Abstract

Recently, breast cancer resistance protein (BCRP/ABCG2) has been shown to limit the oral absorption of its substrates in the intestine. The purpose of this study was to examine whether excipients can be used as inhibitors of BCRP, to improve the oral drug absorption of BCRP substrates. In wild-type mice, Pluronic P85 and Tween 20, given orally 15 min before topotecan administration, increased the area under the plasma concentration-time curve (AUC) of topotecan after oral administration (2.0- and 1.8-fold, respectively). In contrast, Pluronic P85 and Tween 20 were less effective (no significant difference) on the AUC of topotecan after oral administration in Bcrp (-/-) mice (1.2- and 1.2-fold, respectively). Pluronic P85 and Tween 20 given orally did not affect significantly the AUC of topotecan after intravenous administration in wild-type and Bcrp (-/-) mice. Moreover, we determined the mucosal-to-serosal absorptive transport of topotecan using everted mouse ileum. Pluronic P85 and Tween 20 significantly increased the intestinal absorption rate of topotecan in everted sacs from wild-type mice, while, in everted sacs from Bcrp (-/-) mice, the absorption rate was 2.1-fold greater than that in wild-type mice, and these excipients were not significantly effective. There was no significant difference in the intestinal P-gp expression and serosal-to-mucosal secretory transport of rhodamine 123, a typical P-gp substrate. Taken together, these results suggest that Pluronic P85 and Tween 20 can improve the oral bioavailability of BCRP substrates by inhibiting BCRP function in the small intestine.

Introduction

It is well known that active efflux in the intestinal epithelium by ATP-dependent multidrug efflux transporters, such as P-glycoprotein (P-gp), is one of the mechanism limiting oral absorption of drugs, and thus, inhibition of active efflux is one of the strategies used to improve oral absorption of drugs that are pumped out from the intestinal epithelium into the lumen by efflux transporter systems (van Asperen et al., 1997; Meerum Terwogt et al., 1998). During the past two decades, several inhibitors of efflux transporters have been developed to enhance the bioavailability of their substrates (Hyafil et al., 1993; Dantzig et al., 1996; Vincent et al., 2001; Stewart et al., 2004). Although effective in animal experiments, their clinical applicability has been limited (Breedveld et al., 2006) and, consequently, more effective and safer inhibitors are needed.

Excipients, such as surfactants, are extensively used in 'passive' pharmaceutical formulations to improve dissolution of poorly soluble drugs. Recently, it has been found that several excipients can inhibit P-gp. Nerurkar et al. reported that Cremophor EL and Tween 80 enhanced the absorptive transport of a model peptide by inhibiting the secretory directed transport of this peptide in Caco-2 cells (Nerurkar et al., 1996). Yu et al. also reported that vitamin E-TPGS inhibited the efflux system and enhanced the permeability of amprenavir in Caco-2 cells (Yu et al., 1999). Lo reported that Tween 20 markedly enhanced the intracellular accumulation of epirubicin in Caco-2 cells, and enhanced mucosal-to-serosal absorption of epirubicin in the rat jejunum and ileum (Lo, 2003). Previous reports suggest that

coadministration of excipients enhanced oral absorption of P-gp substrates due not only to improved solubilization of drugs, but also inhibition of P-gp-mediated efflux (Yu et al., 1999; Martin-Facklam et al., 2002; Varma and Panchagnula et al., 2005). These excipients are commonly used in human pharmaceutical formulations and are considered safe and relatively non-toxic. Therefore, the use of excipients as the inhibitors of efflux transporters may lead to important pharmacotherapeutic benefits.

Breast cancer resistance protein (BCRP/*ABCG2*) belongs to the ATP-binding cassette (ABC) family of drug efflux transporters (Doyle et al., 1998; Allen and Schinkel, 2002). BCRP is expressed in various normal tissues, such as liver, kidney, brain, placenta, intestine and colon (Allen and Schinkel, 2002; van Herwaarden and Schinkel, 2006). BCRP shows broad substrate specificity including anticancer drugs, food carcinogen, antibiotics, HMG-CoA reductase inhibitors and conjugated metabolites, and can act as an active secretion system by transporting substrates from the cells (Jonker et al., 2000; Litman et al., 2000; Suzuki et al., 2003; van Herwaarden et al., 2003; Hirano et al., 2005; Merino et al., 2005). In the gastrointestinal tract, BCRP is localized in the apical membrane of the intestinal epithelia, and shows quite high levels of mRNA compared with other ABC transporters, including P-gp in the human intestine (Jonker et al., 2000; Maliepaard et al., 2000; Taipalensuu et al., 2001). It has been reported that BCRP has a marked effect on the oral bioavailability of its substrates. The oral availability of topotecan, 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine, ME3277 and nitrofurantoin, typical substrates of BCRP, was dramatically reduced by BCRP (Jonker et al., 2002; van Herwaarden et

al., 2003; Kondo et al., 2005; Merino et al., 2005). BCRP also has been shown to extrude glucuronide and sulfate conjugates formed in enterocytes into the intestinal lumen (Adachi et al., 2005). Furthermore, concomitant use of GF120918, a BCRP and also P-gp inhibitor, and topotecan effectively increased the oral bioavailability of topotecan in a clinical study (Kruijtzer et al., 2002). This cumulative evidence suggests that inhibition of BCRP will improve the oral absorption of drugs.

The purpose of this study was to examine whether excipients could inhibit BCRP function and improve the oral absorption of its substrates. We used Pluronic P85 and Tween 20 as test excipients. Pluronic P85 consists of hydrophilic ethylene oxide and hydrophobic propylene oxide blocks, and Tween 20 contains a sorbitan segment between the polyoxyethylene and fatty acid groups. Both excipients are acceptable for pharmaceutical use as solubilizing agents, suspending agents and emulsifying agents and inhibit P-gp function (Batrakova et al., 2001a; Lo, 2003). In this study, we examined their effect on the oral absorption of topotecan as a typical BCRP substrate drug, because its oral absorption is limited by BCRP (Jonker et al., 2000; Jonker et al., 2002).

Materials and Methods

Materials. Pluronic P85 was kindly gifted by BASF Co. (Parispany, NJ). Tween 20 was purchased from Sigma-Aldrich Co. (St. Louis, MO). Topotecan was kindly provided by GlaxoSmithKline (Uxbridge, Middlesex, UK). Rhodamine 123 was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals used in the experiments were of analytic grade.

Animals. Female Bcrp (-/-) mice and age-matched wild-type control mice were produced as reported previously (Jonker et al., 2002). All mice (8 - 20 weeks) were housed in rooms maintained at 23 ºC and 55 $\pm$ 5 % relative humidity, and allowed free access to food and water during the acclimatization period. The animal work was performed at Hoshi University and complied with the regulations of the Committee on Ethics in the Care and Use of Laboratory Animals.

***In vivo* oral and intravenous administration of topotecan.** Oral and intravenous administration of topotecan was performed as described previously (Jonker et al., 2000). Pluronic P85 and Tween 20 were dissolved in phosphate-buffered saline (PBS; 137 mM NaCl, 2.6 mM KCl, 6.4 mM Na_2HPO_4, and 1.4 mM KH_2PO_4, pH 7.4). Animals, lightly anesthetized with ether, were given Pluronic P85 (100, 250 or 500 mg/kg; 10 µl of drug solution/g body weight), Tween 20 (50, 100 or 250 mg/kg; 10 µl of drug solution/g body weight) or

corresponding amount of buffer by a sonde needle into the stomach. At 15 min after oral

administration of Pluronic P85 and Tween 20, mice received topotecan orally or intravenously

at a dose of 1.0 mg/kg body weight (5 µl of drug solution/g body weight). For intravenous

administration, topotecan was injected into the tail vein of mice lightly anesthetized with ether.

Blood was collected from the tail vein at predetermined time intervals. Plasma was separated by

centrifugation at 13000 ×g for 1 min and kept under refrigeration until analysis. Heparinized

plasma was mixed with seven volumes of methanol. The total topotecan levels (lactone plus

carboxylate form) in plasma were determined by high-pressure liquid chromatography (HPLC)

with fluorescence detection (RF-10AXL, Shimadzu Science East Co., Tokyo, Japan) as

described previously (Rosing et al., 1999). Topotecan was detected using a fluorescence detector

at an excitation wavelength of 380 nm and an emission wavelength of 520 nm. The area under

the plasma concentration-time curve (AUC) from 0 to 4 hours was calculated by the linear

trapezoidal rule.

Preparation of everted sacs. Everted sacs were prepared by a modification of the procedure

described previously (Sakamoto et al., 2005). Mice were anesthetized with ether and sacrificed

by exsanguination from the abdominal aorta. Then, the jejunum and ileum were immediately

removed and rinsed in ice-cold Krebs Ringer-Henseleit bicarbonate buffer (118 mM NaCl, 4.75

mM KCl, 2.50 mM $CaCl_2$, 1.19 mM KH_2PO_4, 1.19 mM $MgSO_4$, and 25 mM $NaHCO_3$, pH 6.5).

Five-cm segments of intestine were isolated to perform the intestinal absorption and intestinal

secretion studies. The intestinal segments were everted using a stainless-steel rod. For the

absorptive transport (mucosal-to-serosal) study, polyethylene tubes were inserted into both ends

of the everted segments and tied. For the secretory transport (serosal-to-mucosal) study, one end

of the everted segment was ligated with silk thread, and a polyethylene tube was inserted into

the other end and tied.

***In vitro* transport study in everted sacs.** For absorptive transport (mucosal-to-serosal) studies

of topotecan, 5 cm segments of the everted ileum were placed in 50 ml Krebs Ringer-Henseleit

bicarbonate buffer gassed with O_2/CO_2 (95:5) at 37 ºC. The everted ileum was initially filled

with 1.5 ml of Krebs Ringer-Henseleit bicarbonate buffer and perfused with the buffer at 0.1

ml/min using an infusion pump (syringe infusion pump; KD Scientific Inc., Holliston, MA)

throughout the transport study. After pre-incubation in the buffer for 15 min, topotecan was

added to the mucosal side to give a final concentration of 10 μM. Then, the outflow perfusate

was collected for 5 min at 15 min intervals up to 90 min. The length of the ileal segment was

measured at the end of the experiments. The concentration of total topotecan in the outflow

perfusate was measured by HPLC. The absorption rate of topotecan in the everted sac was

determined according to the following equation:

$$\text{Absorption rate} = C_{out} \times Q/L \qquad (1)$$

where C_{out} represents the topotecan concentration in the outflow solutions, Q is the perfusion rate (0.1 ml/min), and L is the length of the intestinal segments.

The absorption rates of topotecan in the absence and presence of Pluronic P85 or Tween 20 were also determined by using the above-mentioned absorption transport method. In this experiment, the everted ileum was pre-incubated in 50 ml Krebs Ringer-Henseleit bicarbonate buffer containing 20 µM Pluronic P85 or 250 µM Tween 20 for 2 h at 37 ℃, and then topotecan was added to the mucosal side to give a final concentration of 10 µM. During the experiment, the everted sac was perfused with Krebs Ringer-Henseleit bicarbonate buffer at 0.1 ml/min. The absorption rates of topotecan in the absence and presence of the excipients were determined from the concentration of total topotecan in the outflow perfusate at 60 min.

For the secretory transport (serosal-to-mucosal) studies of rhodamine 123, 5 cm segments of everted jejunum and ileum were placed in 10 ml Krebs Ringer-Henseleit bicarbonate buffer gassed with O_2/CO_2 (95:5) at 37 ℃. After preincubation in Krebs Ringer-Henseleit bicarbonate buffer for 15 min, rhodamine 123 (1 µM; 70 µl/cm tissue) was added to the serosal side. Aliquots (100 µl) of mucosal medium were collected at designated times and then replaced by the same volume of Krebs Ringer-Henseleit bicarbonate buffer. The length of the segment was measured at the end of the experiments. The concentration of rhodamine 123 in mucosal medium was determined by fluorometry (Mithras LB940, Bertold Japan Co. Ltd., Tokyo, Japan). The secretion rate of rhodamine 123 in the everted sac was determined according to the following equation:

$$\text{Secretion rate} = \mathrm{d}C_{\mathrm{m}}/\mathrm{d}t/L \qquad\qquad (2)$$

where C_{m} represents the concentration of rhodamine 123 in mucosal medium, and L is the

length of the intestinal segments.

Western Blot Analysis. For Western blot analysis, crude membrane was prepared from mouse

intestine as described previously (Ogihara et al., 1996). The crude membrane was suspended in

PBS, then frozen in liquid N_2 and stored at -80 ºC until analysis. The protein concentrations in

the crude membrane vesicles prepared from mouse intestine were determined by the method of

Lowry with bovine serum albumin as a standard. The membrane fraction was dissolved in 3 ×

SDS sample buffer (New England Biolabs, Beverly, MA) with β-mercaptoethanol and loaded

onto a 7 % (P-gp or villin) or 8.5 % (Bcrp) SDS-polyacrylamide electrophoresis gel with a

3.75 % stacking gel. The molecular weight was determined using a prestained protein marker

(New England Biolabs). Proteins were transferred electrophoretically to a polyvinylidene

difluoride membrane (Pall, East Hills, NY) using a blotter (Trans-blot; Bio-Rad, Hercules, CA)

at 15 V for 1 h. The membrane was blocked with Tris-buffered saline with 0.05 % Tween 20

(TTBS) and 5 % skimmed milk overnight at 4 ºC. After washing with TTBS, the membrane was

incubated at room temperature for 1 h in TTBS with 100-fold diluted monoclonal antibody

against P-gp (C219; Signet Laboratories, Inc., Dedham, MA), 500-fold diluted monoclonal

antibody against Bcrp (BXP53; Signet Laboratories, Inc.), or 100-fold diluted monoclonal

antibody against villin (C19; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). For the detection

of each protein, the membrane was placed in contact with 500-fold diluted anti-mouse IgG

(P-gp), or 500-fold diluted anti-rat IgG (Bcrp), or 500-fold diluted anti-goat IgG (villin)

conjugated with the horseradish peroxidase (Amersham Biosciences Inc., Piscataway, NJ) for 1

h in TTBS. The band was detected using an ECL Plus Western blotting starter kit (Amersham

Biosciences Inc.), and its intensity was quantified in a luminescent image analyzer (LAS-3000

mini; Fuji Film Co., Tokyo, Japan).

Statistical Analysis. Results are presented as the means ± standard deviation. For group

comparisons, an analysis of variance (ANOVA) with a one-way layout was applied. Significant

differences in the mean values were evaluated by the Student's unpaired t-test or Dunnett's test

for multiple comparison. A p value of less than 0.05 was considered significant.

Results

Effect of different oral doses of Pluronic P85 and Tween 20 on oral topotecan absorption in wild-type mice.

After administration of different doses of Pluronic P85 and Tween 20 orally to wild-type mice 15 min before oral administration of topotecan, the plasma concentration of topotecan was determined at designated times (Fig. 1 and Table 1). Pluronic P85 given orally increased the AUC of topotecan on increasing the dose of Pluronic P85 up to 250 mg/kg. Increasing the Pluronic P85 dose to 500 mg/kg reduced the AUC of topotecan compared with the dose of 250 mg/kg. Similarly, Tween 20 given orally increased the AUC of topotecan on increasing the dose of Tween 20 up to 100 mg/kg, whereas a further increase in the dose of Tween 20 reduced the AUC of topotecan. In both 250 mg/kg Pluronic P85 and 100 mg/kg Tween 20-treated mice, the AUC of topotecan was 2.0-fold higher than that in non-excipient-treated mice.

Effect of oral doses of Pluronic P85 and Tween 20 on intestinal absorption of topotecan in wild-type and Bcrp (-/-) mice.

After administration of 250 mg/kg Pluronic P85 or 100 mg/kg Tween 20 orally to wild-type and Bcrp (-/-) mice 15 min before oral administration of topotecan, the plasma concentration of topotecan was determined at designated times (Fig. 2, Fig. 3 and Table 2). In Pluronic P85-treated wild-type mice, the AUC of topotecan was statistically higher than that in non-excipient-treated wild-type mice (2.0-fold). In contrast, no significant effect of Pluronic

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P85 on the AUC of topotecan was observed in Bcrp (-/-) mice. Similarly, in Tween 20-treated

wild-type mice, the AUC of topotecan was statistically higher than that in non-excipient-treated

wild-type mice (1.8-fold), whereas no significant effect of Tween 20 on the AUC of topotecan

was observed in Bcrp (-/-) mice.

Effect of oral doses of Pluronic P85 and Tween 20 on topotecan pharmacokinetics in wild-type and Bcrp (-/-) mice after intravenous administration.

After administration of 250 mg/kg Pluronic P85 or 100 mg/kg Tween 20 orally to

wild-type and Bcrp (-/-) mice 15 min before intravenous administration of topotecan, the plasma

concentration of topotecan was determined at designated times (Fig. 2, Fig. 3 and Table 2). No

significant effect of Pluronic P85 on the AUC of topotecan was observed in wild-type and Bcrp

(-/-) mice. Similar to Pluronic P85, no significant effect of Tween 20 on the AUC of topotecan

was observed in wild-type and Bcrp (-/-) mice.

Intestinal absorptive transport of topotecan in everted sacs of wild-type and Bcrp (-/-) mice.

The intestinal absorption rate of topotecan was measured using everted sacs, and

compared between wild-type and Bcrp (-/-) mice. Topotecan was applied to the mucosal side,

and the serosal side of the sacs was perfused with drug-free buffer. The concentration of

topotecan in the outflow was determined. Compared with wild-type mice, the intestinal

absorption rate of topotecan was higher in Bcrp (-/-) mice. The intestinal absorption rates of topotecan in wild-type and Bcrp (-/-) mice reached a plateau at 45 min during constant infusion (Fig. 4 (A)). The intestinal absorption rates of topotecan at 60 min, at which steady-state had been reached, in wild-type and Bcrp (-/-) mice were 0.43 ± 0.08 and 0.83 ± 0.14 pmol/min/cm tissue, respectively. The steady-state intestinal absorption rate of topotecan in Bcrp (-/-) mice was statistically higher than that in wild-type mice (2.1-fold; $P<0.01$).

Effect of Pluronic P85 and Tween 20 on intestinal absorptive transport of topotecan in everted sacs of wild-type and Bcrp (-/-) mice.

The steady-state intestinal absorption rate of topotecan in the presence of 20 µM Pluronic P85 or 250 µM Tween 20 was determined (Fig. 4 (B)). Each excipient was added to the mucosal side. Twenty µM Pluronic P85 statistically increased the intestinal absorption rate of topotecan in wild-type mice (2.1-fold), whereas no significant effect of Pluronic P85 was observed in Bcrp (-/-) mice. Similarly, 250 µM Tween 20 statistically increased the intestinal absorption rate of topotecan in wild-type mice (2.5-fold), whereas no significant effect of Tween 20 was observed in Bcrp (-/-) mice.

P-gp expression and transport function in the intestine of wild-type and Bcrp (-/-) mice.

Following Western blot analysis, no significant difference in P-gp expression in jejunum was observed between wild-type and Bcrp (-/-) mice. In the ileum, the P-gp expression in Bcrp

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(-/-) mice slightly increased compared with that in wild-type mice (Fig. 5 (A)). In contrast, Bcrp

was detected in the jejunum and ileum of wild-type mice, but not in Bcrp (-/-) mice (Fig. 5 (A)).

The villin content remained relatively unchanged (Fig. 5 (A)).

The secretory transport of rhodamine 123, a typical P-gp substrate, was measured using

everted sacs of wild-type and Bcrp (-/-) mice. Rhodamine 123 was applied to the serosal side,

and the mucosal efflux of rhodamine 123 was determined. In wild-type and Bcrp (-/-) mice, the

mucosal efflux of rhodamine 123 in the jejunum and ileum was linear up to 60 min (Fig. 5 (B)).

No significant difference was observed in the secretion rates of jejunum and ileum between

wild-type and Bcrp (-/-) mice (Fig. 5 (B)) which were 0.29 ± 0.01 *versus* 0.28 ± 0.06, and $0.64 \pm$

0.16 *versus* 0.64 ± 0.12 pmol/min/cm tissue, respectively.

Discussion

In the present study, to examine whether excipients can be used as inhibitors of BCRP and can improve the oral drug absorption of BCRP substrates, an *in vivo* oral and intravenous topotecan administration study was carried out with an oral dose of Pluronic P85 and Tween 20, and an *in vitro* intestinal topotecan transport study was carried out using wild-type and Bcrp (-/-) mice.

Both Pluronic P85 and Tween 20 significantly increased the AUC of topotecan after oral administration in a dose-dependent manner, but, at a high dose, they were less effective in wild-type mice (Fig. 1 and Table 1). In contrast, neither Pluronic P85 nor Tween 20 given orally affected the AUC of topotecan after intravenous administration in wild-type mice (Fig. 2 and Table 2), implying that these excipients given orally did not affect the systemic clearance of topotecan, probably because of the low absorption of these excipients. These results suggest that excipient-mediated enhancement of the AUC of topotecan after oral administration may be due to an increase in its intestinal absorption. The ability of excipients to enhance oral drug absorption can be possibly ascribed to increasing the solubility of drugs in the intestinal lumen and/or inhibition of efflux transporters (Yu et al., 1999; Varma and Panchagnula, 2005). To investigate the contribution of increasing solubility of drugs and inhibition of BCRP, the effect of Pluronic P85 and Tween 20 was examined in Bcrp (-/-) mice. Neither Pluronic P85 nor Tween 20 exhibited any enhancement of the AUC of topotecan after oral and intravenous administration in Bcrp (-/-) mice (Fig. 3 and Table 2). These results suggest that inhibiting

BCRP by excipients mainly contributes to the enhancement of oral topotecan absorption and

increasing drug solubility was at most minimal. These were further supported by *in vitro* study

using everted mouse intestine. The steady-state intestinal absorption rate of topotecan was

significantly greater in Bcrp (-/-) mice than that in wild-type mice (Fig. 4 (A)). Consistent with

in vivo observation, both Pluronic P85 and Tween 20 significantly increased the steady-state

intestinal absorption rates of topotecan in everted sacs from wild-type mice, while the effect was

reduced in sacs from Bcrp (-/-) mice (Fig. 4 (B)).

Topotecan is also a substrate of P-gp (Chen et al., 1991; Jonker et al., 2000), and it has

been demonstrated that P-gp also plays a significant role in the pharmacokinetics of topotecan

after oral administration (Jonker et al., 2000). Whether the expression and the transport activity

of intestinal P-gp is changed due to the impairment of Bcrp activity remains in question. This

was investigated by Western blot analysis, and an *in vitro* transport study using everted sacs.

Western blot analysis showed that P-gp expression was similar in the jejunum, but there was a

slight increase in the ileum of Bcrp (-/-) mice (Fig. 5 (A)). There was no significant change in

the transport function of P-gp between wild-type and Bcrp (-/-) mice (Fig. 5 (B)). Namely, there

was no significant difference in the intestinal P-gp transport function between wild-type and

Bcrp (-/-) mice. Therefore, it can be concluded that the increase in the AUC of topotecan after

oral administration in Bcrp (-/-) mice is due to impairment of Bcrp in the intestine.

Taken together, the present study elucidated that Pluronic P85 and Tween 20 inhibit

intestinal efflux by Bcrp, and can improve the oral absorption of topotecan. The mechanism

underlying the inhibition of BCRP by these excipients remains unknown. Previous reports have suggested that a change in the microviscosity of the plasma membrane due to integration of excipients, and/or a reduction in the intracellular ATP are the underlying mechanisms (Dudeja et al., 1995; Batrakova et al., 2001a; Hugger et al., 2002). However, the effect of a change in the microviscosity remains questionable since cholesterol and benzylalcohol, known as membrane fluidity modulators, did not affect the efflux transporters (Rege et al., 2002). Additionally, although Pluronic P85 has been shown to inhibit P-gp and reduce the intracellular ATP, there is no report of other excipients examining the reduction in intracellular ATP. It is likely that these excipients may competitively inhibit substrate binding to the efflux transporter, although further studies are needed to clarify the mechanism.

For clinical application, it should be noted that there was an optimal oral dose of excipients in order to enhance the oral topotecan absorption (Fig. 1 and Table 1). Similar tendencies have been reported from in vitro drug transport studies (Nerurkar et al., 1996; Batrakova et al., 1999; Yu et al., 1999; Rege et al., 2002; Shono et al., 2004; Varma and Panchagnula, 2005). Possibly, this is due to the micelle formation of the excipients at the highest doses. Incorporation of topotecan into the micelle might reduce the effective concentration of topotecan in the intestinal lumen, thus, no enhancement of oral topotecan absorption might be observed. Therefore, doses of excipients, such as surfactants need to be designed achieve their appropriate concentration in the intestine in order to improve oral drug absorption. In addition, if excipients are administered intravenously, they might increase the penetration of drugs into

the brain by inhibiting efflux transporters in the blood-brain barrier. In fact, it has been reported

that the intravenous coadministration of Pluronic P85 with digoxin markedly increases the

penetration of digoxin into the brain by inhibiting P-gp function at this critical point (Batrakova

et al., 2001b). Thus, an increase in the accumulation of drugs in the brain by intravenous

coadministration of excipients might lead to toxicity.

In conclusion, this study demonstrated that the excipients Pluronic P85 and Tween 20 can

improve the oral bioavailability of topotecan, a BCRP substrate, by inhibiting BCRP function in

the small intestine. Thus, some excipients are not as inert as initially thought but are indeed

functional.

Acknowledgments

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Footnotes

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Legends for figures

Fig. 1. Time-profiles of the plasma concentration of topotecan in mice given different oral doses of Pluronic P85 or Tween 20.

Mice were given an oral dose of Pluronic P85 (100, 250 and 500 mg/kg) (A) or Tween 20 (50, 100 and 250 mg/kg) (B), 15 min before an oral dose of topotecan (1 mg/kg). The plasma concentration of topotecan was determined at designated times. Each point and bar represents the mean ± S.D. (n = 3-6). Where vertical bars are not shown, the S.D. was within the size limit of the symbol. * $p < 0.05$; ** $p < 0.01$, significant difference between with and without excipients.

Fig. 2. Time-profiles of the plasma concentration of topotecan after oral and intravenous topotecan administration in wild-type mice given an oral dose of Pluronic P85 or Tween 20.

Wild-type mice were given an oral dose of Pluronic P85 (250 mg/kg) or Tween 20 (100 mg/kg), 15 min before an oral (A) or intravenous (B) dose of topotecan (1 mg/kg). The plasma concentration of topotecan was then determined at designated times. Each point and bar represents the mean ± S.D. (n = 3). Where vertical bars are not shown, the S.D. was within the size limit of the symbol. * $p < 0.05$; ** $p < 0.01$, significant difference between with and without excipients.

Fig. 3. Time-profiles of the plasma concentration of topotecan after oral and intravenous

topotecan administration in Bcrp (-/-) mice given an oral dose of Pluronic P85 or Tween 20.

Bcrp (-/-) mice were given an oral dose of Pluronic P85 (250 mg/kg) or Tween 20 (100 mg/kg),

15 min before an oral (A) or intravenous (B) dose of topotecan (1 mg/kg). The plasma

concentration of topotecan was then determined at designated times. Each point and bar

represents the mean ± S.D. (n = 3). Where vertical bars are not shown, the S.D. was within the

size limit of the symbol. * $p < 0.05$; ** $p < 0.01$, significant difference between with and

without excipients.

Fig. 4. Intestinal absorption of topotecan using everted sacs. (A) Absorption rate of topotecan in

the ileum of wild-type and Bcrp (-/-) mice. Segments of ileum from wild-type and Bcrp (-/-)

mice were everted to prepare the sacs. Topotecan was applied to the mucosal side medium to

give a final concentration of 10 µM. The serosal side of the sacs was perfused with drug-free

buffer, and the concentrations of topotecan in the outflow were determined. The absorption rate

was defined by eq. 1, as described under *Materials and Methods*. Each point and bar represents

the mean ± S.D. (n = 3). * $p < 0.05$; ** $p < 0.01$, significant difference from wild-type mice. (B)

Effect of excipients on the absorption rates of topotecan in everted sacs of wild-type and Bcrp

(-/-) mice. The absorption rates of topotecan were determined at 60 min in the absence and

presence of Pluronic P85 (20 µM) or Tween 20 (250 µM). Each point and bar represents the

mean ± S.D. (n = 3). * $p < 0.05$; ** $p < 0.01$, significant difference between with and without

excipients in the same animals. [##] $p < 0.01$, significant difference between wild-type and Bcrp

(-/-) mice given the same treatment.

Fig. 5. Expression level and transport function of intestinal P-gp in wild-type and Bcrp (-/-) mice. (A) Western blot analysis of P-gp, Bcrp and villin expression in the jejunum and ileum of wild-type and Bcrp (-/-) mice. Crude membrane fractions (10 µg of protein per lane) from mouse jejunum and ileum were subjected to Western blot analysis. WT, KO, Jej and Ile represent wild-type mice, Bcrp (-/-) mice, jejunum and ileum, respectively. (B) Intestinal transport activity in wild-type and Bcrp (-/-) mice. The everted sacs of jejunum and ileum of wild-type and Bcrp (-/-) mice were incubated, and rhodamine 123 (1 µM) was added to the serosal side. The concentration of rhodamine 123 in mucosal medium was determined. Each point and bar represents the mean $\pm$ S.D. (n = 3).

TABLE 1

Area under the plasma concentration-time curve of wild-type mice after oral topotecan administration with or without different oral doses of Pluronic P85 or Tween 20

	AUC oral (h • µg/l)
- excipients	**41.2 ± 13.6**
+ Pluronic P85	
100 mg/kg	**54.7 ± 5.4**
250 mg/kg	**82.0 ± 26.3** *
500 mg/kg	**58.0 ± 15.0**
+ Tween 20	
50 mg/kg	**62.9 ± 17.5**
100 mg/kg	**82.1 ± 18.1** *
250 mg/kg	**53.5 ± 20.5**

Mice receiving topotecan (1 mg/kg) orally were given an oral dose of Pluronic P85 (100, 250 or 500 mg/kg) or Tween 20 (50, 100 or 250 mg/kg). The plasma concentration of topotecan was then determined. Results are expressed as means ± S.D. (n = 3-6). * $p < 0.05$, significant difference between with and without excipients by ANOVA followed by Dunnett's test. Data were taken from Figure 1.

TABLE 2

Area under the plasma concentration-time curve of wild-type and Bcrp (-/-) mice after oral and intravenous topotecan administration with or without an oral dose of Pluronic P85 or Tween 20

	AUC oral (h • µg/l)			AUC i.v. (h • µg/l)		
Wild-type mice						
- excipients	56.5	±	12.0	458	±	97
+ Pluronic P85	112	±	27 *	517	±	95
+ Tween 20	102	±	25 *	606	±	86
Bcrp (-/-) mice						
- excipients	206	±	29 #	785	±	76 ##
+ Pluronic P85	244	±	65 #	805	±	79 #
+ Tween 20	237	±	28 ##	888	±	136 #

Mice receiving topotecan (1 mg/kg) orally or intravenously were given an oral dose of Pluronic P85 (250 mg/kg) or Tween 20 (100 mg/kg). The plasma concentration of topotecan was then determined. Results are expressed as means ± S.D. (n = 3-6). * $p < 0.05$, significant difference between with and without excipients in the same animals by ANOVA followed by Dunnett's test. # $p < 0.05$; ## $p < 0.01$, significant difference between wild-type and Bcrp (-/-) mice given the same dose of excipients by ANOVA followed by Student's *t*-test. Data were taken from Figures 2 and 3.
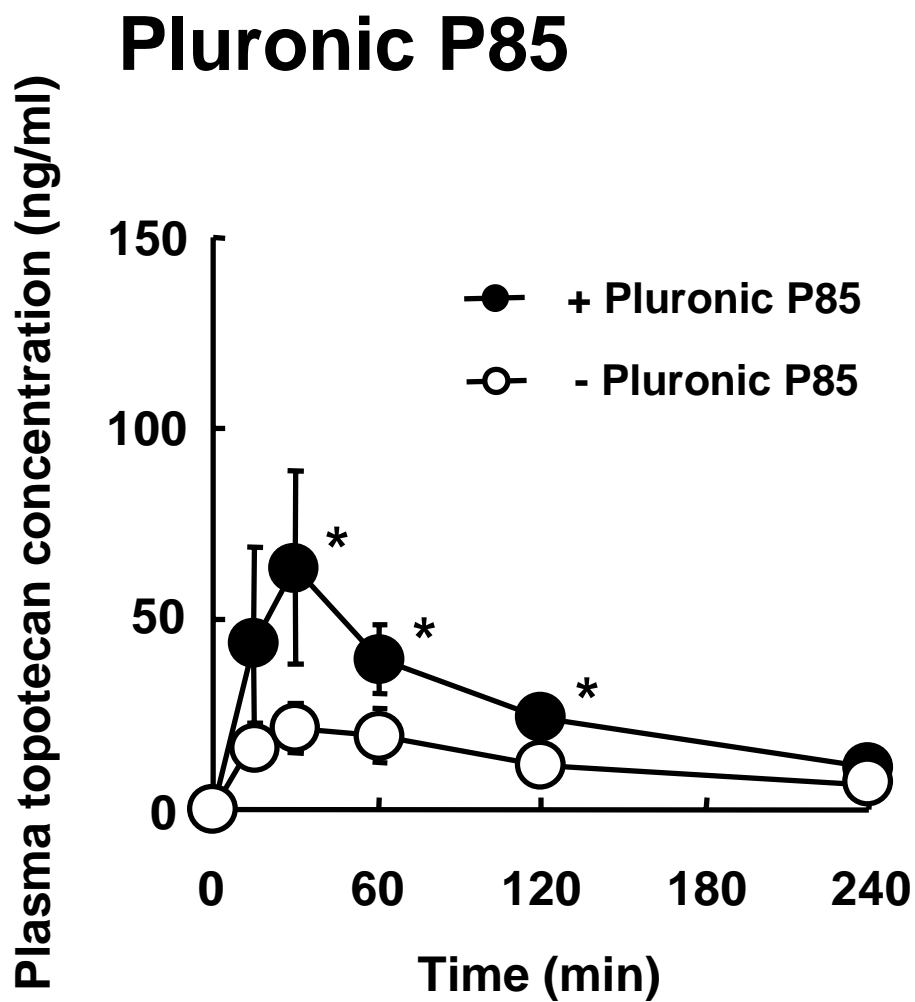
Fig. 1.

(A) Pluronic P85

(B) Tween 20
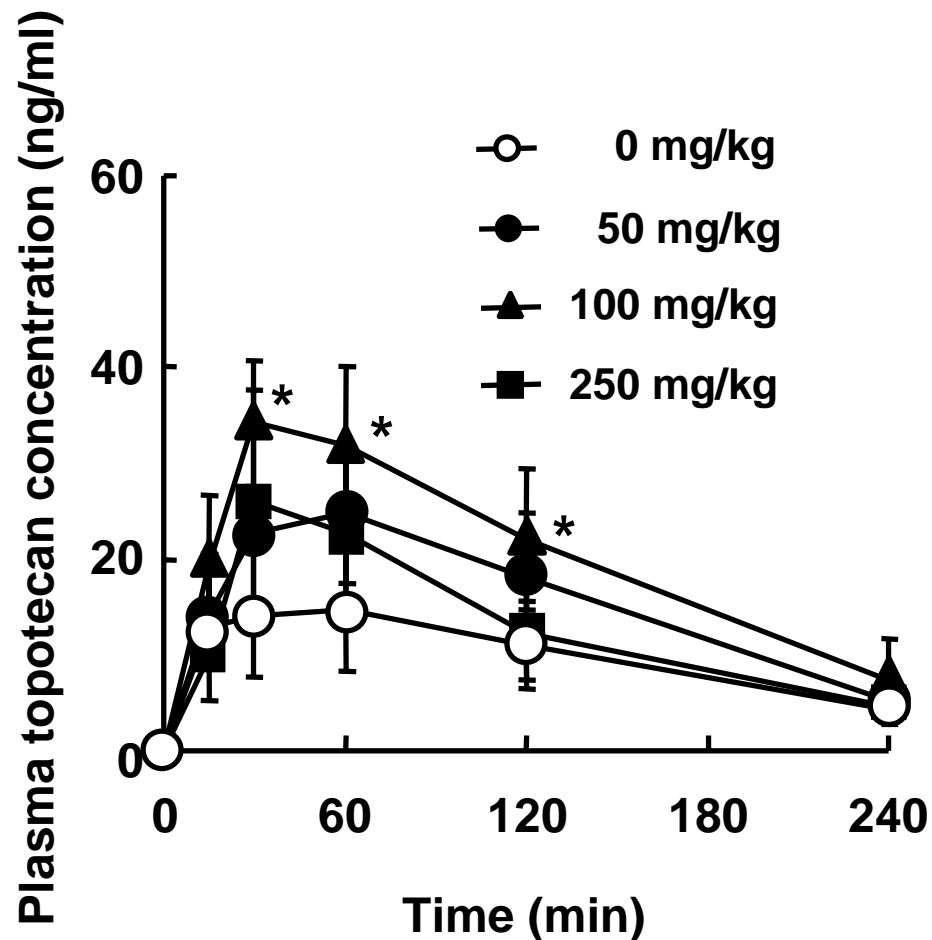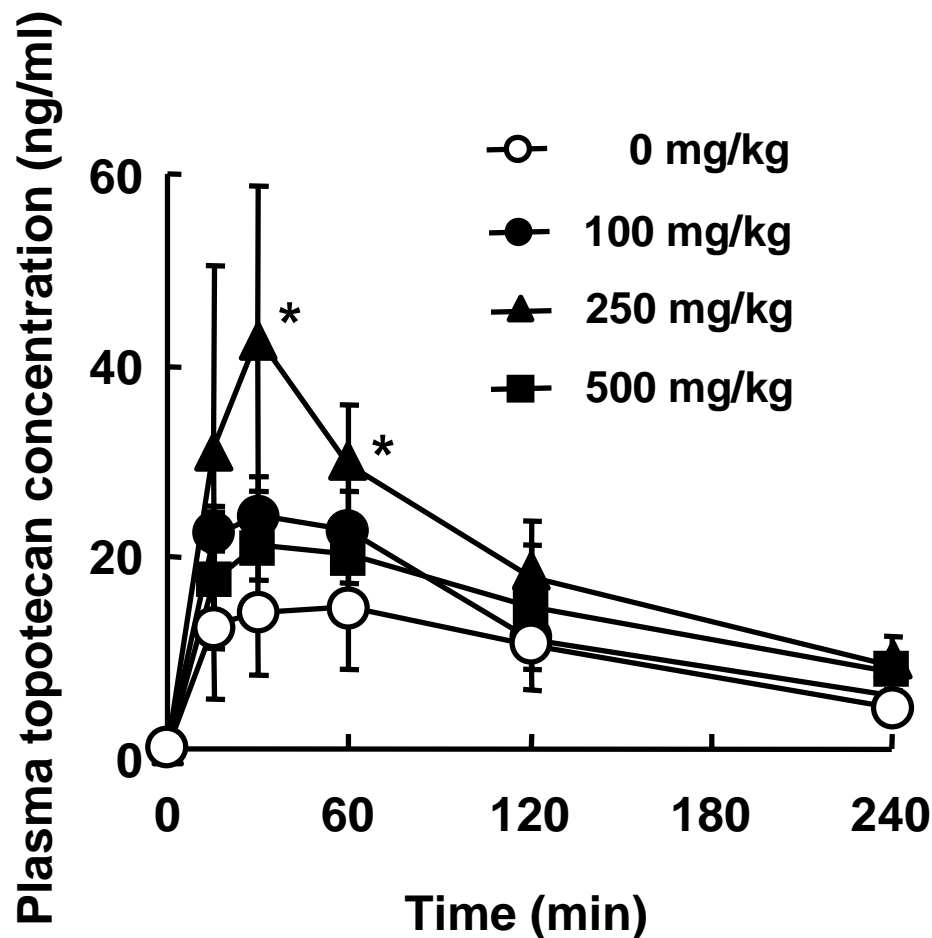
Fig. 2. (A)

(A)

Fig. 2. (B)

(B)

Fig. 3. (A)

(A)

Fig. 3. (B)

(B)

Pluronic P85

Y-axis: Plasma topotecan concentration (ng/ml)
Values: 0, 200, 400, 600, 800, 1000, 1200

Legend:
● + Pluronic P85
○ - Pluronic P85

X-axis: Time (min)
Values: 0, 60, 120, 180, 240

Tween 20

Y-axis: Plasma topotecan concentration (ng/ml)
Values: 0, 500, 1000, 1500, 2000

Legend:
▲ + Tween 20
△ - Tween 20

X-axis: Time (min)
Values: 0, 60, 120, 180, 240

Fig. 4. (A)

(A)

Fig. 4. (B)

Fig. 5. (A)

(A)

Fig. 5. (B)

(B)

Jejunum

Ileum