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Research Paper

Effect of poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) micelles on pharmacokinetics and intestinal toxicity of irinotecan hydrochloride: potential involvement of breast cancer resistance protein (*ABCG2*)

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Abstract

Objectives Intestinal toxicity and low levels of systemic drug exposure are among the major problems associated with tumour therapy. We have developed poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (PEO-PPO-PEO) micelles loaded with irinotecan hydrochloride (CPT-11) hoping to decrease CPT-11-induced intestinal toxicity while increasing its systemic exposure. In addition, we have investigated the potential involvement of breast cancer resistance protein (BCRP) in biliary excretion, pharmacokinetics, and intestinal toxicity of CPT-11.

Methods PEO-PPO-PEO micelles were prepared using PEO_{20}-PPO_{70}-PEO_{20} and lecithin. The effect of PEO-PPO-PEO micelles on BCRP-mediated cellular accumulation and transport efflux of CPT-11 was evaluated in MDCKII/BCRP cells. The biliary excretion, intestinal damage, and pharmacokinetic study of CPT-11-loaded PEO-PPO-PEO micelles were investigated in rats.

Key findings The obtained micelles could effectively inhibit BCRP-mediated CPT-11 efflux in MDCKII/BCRP cells, and significantly decrease the drug biliary excretion in rats. Moreover, intestinal toxicity, assessed by microscopic examination of pathological damage, was ameliorated in rats injected with PEO-PPO-PEO micelles compared with rats injected with CPT-11 alone. Treatment with PEO-PPO-PEO micelles resulted in prolonged circulation time in blood and increased bioavailability of CPT-11 and SN-38 (7-ethyl-10-hydroxycamptothecin).

Conclusions PEO-PPO-PEO micelles were identified as promising carriers able to reduce intestinal toxicity and increase antitumour therapeutic effect of CPT-11. The study indicated a potential involvement of BCRP in CPT-11 pharmacokinetics and CPT-11-induced intestinal toxicity.

Keywords biliary excretion; breast cancer resistance protein (BCRP); intestinal toxicity; irinotecan hydrochloride (CPT-11); poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) micelles

Introduction

Irinotecan hydrochloride (CPT-11) is an anticancer agent that exerts its effect by inhibiting DNA topoisomerase I.[1] It is a prodrug that can be hydrolysed to form the active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin) and inactive metabolite SN-38 glucuronide (SN-38G).[2,3] The structure and major metabolic pathways for CPT-11 are outlined in Figure 1. Severe intestinal impairments associated with diarrhoea is a major dose-limiting toxicity of CPT-11 and treatment with conventional antidiarrhoeal agents does not always produce a good response.[4–7] The CPT-11-induced intestinal impairments and diarrhoea are thought to be due to CPT-11 and its metabolites SN-38 and SN-38G excretion into the gastrointestinal tract from the bile duct.[8,9] Thus, inhibition of the biliary excretion of CPT-11 and its metabolites could reduce intestinal toxicity and ameliorate severe diarrhoea.[7]

CPT-11 and a wide variety of antitumour drugs, including mitoxantrone, topotecan and SN-38, are substrates of the breast cancer resistance protein (BCRP, gene symbol *ABCG2*).[10] BCRP is one of the ATP-binding cassette (ABC) transporters located in the apical membrane of enterocytes, canalicular membrane of hepatocytes, placental syncy-

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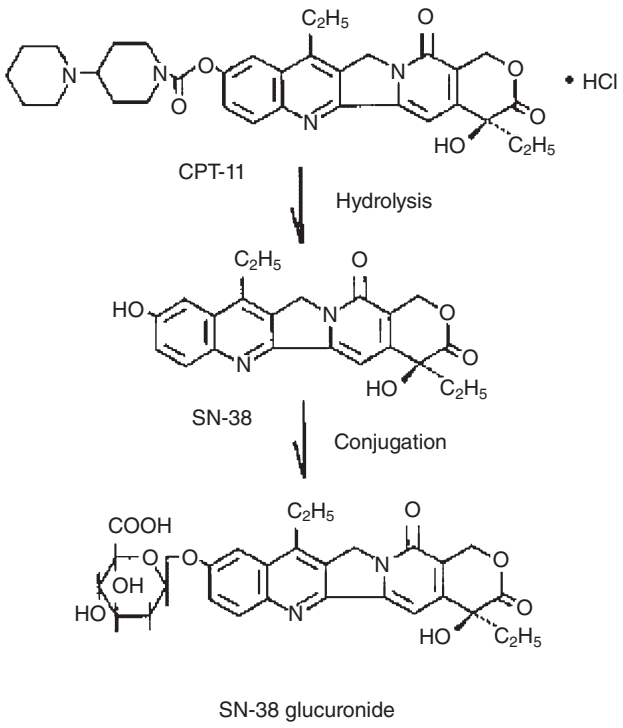

Figure 1 Structure and metabolic pathway of irinotecan hydrochloride. Irinotecan hydrochloride, CPT-11; the active metabolite 7-ethyl-10-hydroxycamptothecin, SN-38; the inactive metabolite SN-38 glucuronide, SN-38G.

tiotrophoblasts, as well as tumour cells, and plays an important role in limiting the intestinal drug absorption and facilitating the efflux of its substrates. Recent studies have shown that biliary excretion of both CPT-11 and SN-38 depends on the presence of drug-transporting proteins expressed on the bile canalicular membrane.[11,12] Particularly, BCRP expressed in liver canalicular membrane plays a non-negligible role in the biliary excretion of its substrate drugs.[13] Previously we reported that biliary excretion of CPT-11 was found to decrease after coadministration of BCRP micromolecular inhibitors and some excipients.[14] However, BCRP micromolecular inhibitors may have undesired pharmacological action, and cause systemic toxicity. The observed inhibitory effect of excipients on BCRP was relatively low. Only administration of large amounts of these excipients could change the drug behaviour *in vivo*, and long-term administration may also cause toxicity.

Nanotechnology holds a tremendous potential for improving efficacy and reducing adverse effects of anticancer agents. Polymer-based nanotechnology has become one of the most attractive and fastest-growing areas of pharmaceutical research. The triblock copolymers poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) (PEO-PPO-PEO) have been used extensively in pharmaceutical formulations to deliver various therapeutic drugs.[15,16] Recent data have indicated that PEO-PPO-PEO triblock copolymers can have more than merely inert carrier functions. In fact, they were shown to inhibit drug efflux transporters, such as P-glycoprotein (P-gp), multidrug resistance proteins (MRPs) and BCRP.[17] PEO-PPO-PEO micelles can release copolymer molecules *in vivo* and, at high concentration, they locally inhibit transporters, thus avoiding systemic toxicity. In addition, nanocarriers with blocks made of poly (ethylene oxide) are sterically stabilized and undergo limited opsonization and uptake by the macrophages of the reticuloendothelial system, allowing the micelles to circulate longer in the blood. Thus, they were shown to improve delivery of antitumour drug to tumours via the enhanced permeability and retention (EPR) effect and to have enhanced antitumour activity.[18]

In this study, we have developed PEO-PPO-PEO micelles to encapsulate CPT-11. PEO-PPO-PEO was exploited both as a nanocarrier and drug efflux transporter inhibitor to increase bioavailability and decrease intestinal toxicity of CPT-11. The inhibitory effect of PEO-PPO-PEO micelles on BCRP-mediated transport of CPT-11 was examined in BCRP overexpressing MDCKII cells. Biliary excretion and pharmacokinetics of micelles was investigated in rats, as well as the potential involvement of BCRP. The intestinal damage caused by the micelles was investigated to evaluate their possible ameliorative effects on CPT-11-induced intestinal toxicity.

Materials and Methods

Materials

CPT-11 and camptothecin were purchased from Junjie Biotechnology Co. Ltd (Shanghai, China). SN-38 was obtained from ZhongShuo Pharmaceutical Technology Development Co. Ltd (Beijing, China). Novobiocin sodium (a specific BCRP inhibitor), β-glucuronidase and macrogol 400 (polyethylene glycol 400; PEG400) were from Sigma-Aldrich (St Louis, MO, USA). The soybean lecithin Epikuron 200 (E200) was obtained from Degussa Food Ingredients GmbH (Essen, Germany), with a phosphatidylcholine content of more than 92%. PEO_{20}-PPO_{70}-PEO_{20} block copolymer was provided by BASF Corp. (Parispany, NJ, USA). The molecular weight of this compound was approximately 5800 Da, and the content of the PEO blocks was approximately 30 wt %.

Animals and cell culture

Male Sprague-Dawley rats (200 ± 20 g) were obtained from the Shanghai Institute of Materia Medica (Shanghai, China). Animals were housed in a room maintained at a temperature of 23 ± 2°C with a relative humidity of 55 ± 15%, and a 12-h light–dark cycle. Food and water were freely available throughout the acclimatization and experimental periods. All experiments were approved by the Institutional Animal Care and Use Committee of Shanghai Institute of Materia Medica.

BCRP overexpressing MDCKII cell line (MDCKII/BCRP) and its corresponding wild type cell line (MDCKII/wt) were kind gifts from Dr A. H. Schinkel (The Netherlands Cancer Institute, Amsterdam, Netherlands). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Tulsa, OK, USA) supplemented with 10% fetal bovine serum (Gibco, USA), penicillin (100 U/ml), and streptomycin (100 μg/ml) (Sigma-Aldrich, USA). Cells were grown at 37°C in a 5% CO_2 / 95% air atmosphere, and given fresh medium every other day.

The integrity of the cell monolayer was evaluated before the transport experiments by measuring transepithelial electrical resistance (TEER) and resorcinol phthalein permeability across the monolayer. MDCKII cell monolayer was considered intact and suitable for transport experiments when TEER value was 130~160 Ω·cm^2 and apparent permeability coefficient (P_{app}) of resorcinol phthalein was 0.2 ~ 2 × 10^{-6} cm/s.[19,20]

Preparation of CPT-11-loaded PEO-PPO-PEO micelles and CPT-11 injection

CPT-11-loaded PEO-PPO-PEO micelles were prepared as follow: E200/PEG400 (1 : 4 w/w) were dissolved in alcohol. The solvent was evacuated in a rotary evaporator (Rotavapor R-210, BUCHI, Switzerland) to form a viscous mixture (E200-PEG400). CPT-11, E200-PEG400 and PEO_{20}-PPO_{70}-PEO_{20} were mixed at 70°C until a uniform melt was formed. Lactic acid solution (0.1%) was slowly added to the melt with agitation by an Ultra Turrax (Ika, Guangzhou, China). The resulting clear micellar solution was filtered through a 0.22 μm filter (Millipore, USA).

CPT-11 injection was prepared according to commercial Camptosar injection instruction. Each milliliter of CPT-11 injection contained 20 mg CPT-11, 45 mg sorbitol, and 0.9 mg lactic acid. The pH of the solution was adjusted to 3.5 with sodium hydroxide.

Characterization of CPT-11-loaded PEO-PPO-PEO micelles

The particle size and size distribution of CPT-11-loaded PEO-PPO-PEO micelles were examined using dynamic light scattering with a Malvern Zetasizer Nano ZS90 (Malvern, UK) at 25°C. The morphological characteristics of the micelles were examined using an atomic force microscope (Dimension 3100, Veeco Metrology Group, Santa Barbara, CA, USA). The micelles were diluted with distilled water and placed on a mica surface. The surface morphology of the micelles was observed after being dried at room temperature.

Entrapment efficiency (EE, %) and drug-loading coefficient (DL, %) were determined using an ultrafiltration–centrifugation technique, and the recovery for this method was validated.[21] The CPT-11-loaded micelles (500 μL) were placed into Microcon YM-10 filter units (10 000 MW, Millipore, USA) and centrifuged at 4000*g* for 15 min. Free CPT-11 not incorporated into micelles was separated in the ultrafiltrate. The content of free or total CPT-11 in the micelles was determined using HPLC. EE and DL were then calculated according to equations 1 and 2, respectively:

$$\text{EE}\,\% = \frac{W_T - W_F}{W_T} \times 100\% \quad (1)$$

$$\text{DL}\,\% = \frac{W_T \times EE\%}{W_M + W_T} \times 100\% \quad (2)$$

where W_T is the weight of total drug in the micelles, W_F is the weight of free drug in the ultrafiltrate after centrifugation, and W_M is the weight of the initial feeding polymer.

CPT-11 cellular accumulation in BCRP overexpressing cells

The accumulation study was carried out as described with minor modifications.[22] MDCKII /wt or MDCKII/BCRP cells were plated onto 28 cm^2 plastic culture dishes at a density of 1 × 10^6 cells/cm^2. CPT-11 (10 μM) in DMEM was added to exponentially growing (approximately 80% confluent) cells. Medium was removed after 2 h, and dishes were rapidly washed five times with ice-cold PBS. To detach the cells for subsequent cell counting 0.25% trypsin-EDTA was used. The cells were suspended in extraction solution (methanol with 1% H_3PO_4) containing camptothecin (used as the internal standard). After vortex-mixing, the cellular suspensions were ultrasonicated and centrifuged at 10 000*g* for 15 min, and CPT-11 concentration in the supernatant was analysed by LC-MS/MS.

For inhibition studies, cells were incubated for 2 h with CPT-11-loaded PEO-PPO-PEO micelles (formulation M3), CPT-11 (10 μM) plus PEO_{20}-PPO_{70}-PEO_{20} (3, 5, 10 and 30 μM), and CPT-11 (10 μM) plus novobiocin (30 μM), and treated as described above. Novobiocin, a specific BCRP inhibitor, was used as the positive control.[23] CPT-11-loaded PEO-PPO-PEO micelles were diluted with DMEM, and the final concentrations of CPT-11 and PEO_{20}-PPO_{70}-PEO_{20} were approximately 10 μM.

CPT-11 transport across BCRP overexpressing cells

MDCKII /wt and MDCKII/BCRP cells were seeded in Corning Transwell filter insert (0.4 μm pore size, 12-mm diameter) and grown at confluence for four days. Both compartments were then pre-incubated in Hank's buffered salt solution (HBSS; containing 10 mM HEPES and 10 mM glucose, pH 7.4, Invitrogen, USA) for 2 h. For the transport experiment, the medium was replaced with HBSS containing CPT-11 (10 μM) on the apical side (A–B) or basolateral side (B–A). Fresh drug-free HBSS was placed on the receiver side. The plates were incubated on an orbital shaker (60 rev/min) at 37°C. The incubation medium (0.35 ml) was drawn from the receiver side at 30, 45, 60, 75, 90 and 105 min, and replaced with fresh HBSS. The concentration of CPT-11 in the samples was measured by LC-MS/MS.

To evaluate the effect of inhibitors and micelles on the directional transport of CPT-11, the experiments were conducted as described above by replacing CPT-11 solution in the donor side with CPT-11-loaded PEO-PPO-PEO micelles (formulation M3), CPT-11 (10 μM) plus PEO_{20}-PPO_{70}-PEO_{20} (5, 10 and 30 μM), and CPT-11 (10 μM) plus novobiocin (30 μM).

The apparent permeability coefficient (P_{app}) is expressed in cm/s and calculated according to equation 3:

$$P_{app} = \frac{dQ}{dt} \times \frac{1}{60} \times \frac{1}{A} \times \frac{1}{Q_0} \quad (3)$$

where dQ/dt is the permeability rate (nmol/min); A is the surface area of the membrane (cm^2); and Q_0 is the initial concentration in the donor chamber (nmol/ml).

Studies of drug biliary excretion

Rats were anaesthetized by a single intraperitoneal injection of 20% ethylurethane solution (approximately 0.6 ml/100 g body weight) without prior fasting. After each rat was completely anaesthetized, the abdomen was opened along the longitudinal midline. The liver was checked for any obvious tumours or signs of inflammation. The common bile duct was visualized and separated. A small incision was made on the anterior wall with microscissors. The common bile duct was then cannulated with a fine cannula (0.50-mm diameter) and secured into position with a cotton thread. Bile samples were collected in tubes. During the experiments, rats were placed in restrictive cages under anaesthesia and their body temperatures were maintained at 37°C.

CPT-11 injection, CPT-11-loaded PEO-PPO-PEO micelles (formulation M3), CPT-11 plus novobiocin (50 mg/kg) or PEO_{20}-PPO_{70}-PEO_{20} (75 mg/kg) were injected intravenously into the tail vein 20 min after bile duct cannulation. The dose of CPT-11 was set at 15 mg/kg. After injection of CPT-11-loaded PEO-PPO-PEO micelles, the blood concentrations of CPT-11 and PEO_{20}-PPO_{70}-PEO_{20}, calculated according to the blood volume of rats, were both approximately 10 μM. The doses of various formulations *in vivo* were similar to those used in in-vitro cellular experiments.

Bile samples were collected every 30 min for 6 h, and their volumes were measured. Before analysis, 10 000 U/ml β-glucuronidase was added to the bile samples, which were then incubated at 37°C for 3 h to ensure the hydrolysis of SN-38G to SN-38. The samples were prepared as described previously.[14]

Pharmacokinetic studies

Male Sprague-Dawley rats were used to investigate the effects of novobiocin, PEO_{20}-PPO_{70}-PEO_{20} and PEO-PPO-PEO micelles on CPT-11 pharmacokinetics after intravenous (i.v.) administration. Rats were randomly divided into four groups and injected in the tail vein with a single 15 mg/kg dose CPT-11, CPT-11 plus novobiocin (50 mg/kg), CPT-11 plus PEO_{20}-PPO_{70}-PEO_{20} (75 mg/kg), or CPT-11-loaded PEO-PPO-PEO micelles (formulation M3). Blood samples were collected into heparinized tubes from the ocular vein at predetermined time intervals after intravenous administration. Blood was immediately processed by centrifugation at 3000*g* for 10 min to separate the plasma. Plasma samples were frozen and stored at -70°C until analysis. Samples were prepared before analysis as follows: 10 μl orthophosphoric acid and 50 μl camptothecin (5 μg/ml) as internal standard were added to 100 μl of each plasma sample and stored away from light at 37°C for 2 h. Subsequently, ice-cold methanol (200 μl/sample) was added, samples were vortexed for 5 min and centrifuged at 10 000*g* for 15 min. The clear supernatant (50 μl) was injected into the HPLC for analysis.

LC-MS/MS and HPLC analysis

The concentrations of CPT-11 in cellular uptake and transport samples were determined using the Thermo Finnigan LTQ mass spectrometer (Thermo Finnigan Corporation, San Jose, CA, USA) operated in positive ionization multiple reaction monitoring mode. The transitions for CPT-11 and camptothecin (used as internal standard) were 587.6→167.2 and 349.3→305.3, respectively. The ionization voltage and collision energy were set at 5000 V and 55 eV. Chromatographic separation was performed using Alltech-Alltima-C18 analytical column (2.1 × 150 mm, 3 μm; Alltech Technologies, Tallahassee, FL, USA) with a mobile phase of H_2O, 1% formic acid (A)–methanol, and 1% formic acid (B) (90 : 10, v/v). The gradient program was set as follows: 0–2 min, B, 10%; 2–4 min, B, 10–90%; 4–6 min, B, 90–90%; 6–8 min, B, 90–10% and 8~10 min, B, 10%. The flow rate was set at 0.2 ml/min, running time for each sample was 10 min, and the injection volume was 10 μl.

The concentrations of CPT-11 and SN-38 in bile and plasma were assayed using reversed-phase HPLC (Agilent 1100 series, USA). The column was a ZORBAX SB-C18 5 μm, 150 × 4.6 mm (Agilent, USA), and the mobile phase consisted of acetonitrile 50 mM dipotassium hydrogen phosphate buffer containing 10 mM sodium 1-octane-sulfonate, with pH adjusted to 3.0 with orthophosphoric acid (26/74, v/v). The mobile phase was delivered at a flow rate of 1.0 ml/min, the column effluent was monitored at 370 nm, and the injection volume was 50 μl.

Validation procedure, including selectivity, limit of quantitation, linearity, precision and accuracy, showed that the LC-MS/MS and HPLC analysis methods had good efficiency for determination of CPT-11 and SN-38.

Histological evaluation of intestinal damage

Sprague-Dawley rats were injected in the tail vein with CPT-11, CPT-11 plus novobiocin sodium (50 mg/kg), or CPT-11-loaded PEO-PPO-PEO micelles (formulation M3) once a day for 10 consecutive days at a dose of 60 mg/kg.

The effects of the micelles and novobiocin on CPT-11-induced intestinal epithelial injury in rats were evaluated by examining histological changes at the microscopic level. The experimental Sprague-Dawley rats were killed by inhalation exposure to CO_2 on day 10, and the intestine (ileum, cecum and colon) was fixed in 10% neutral buffered formaldehyde. After routine processing, the segments were embedded in paraffin wax using a paraffin embedding machine (Thermo Shandon Ltd). Slices of 4 μm were obtained using a rotary microtome (Leica RM2135, Leica Microsystem AG, Wetzlar, Germany), stained with haematoxylin-eosin (H&E) and examined by light microscopy (Leica AS/LMD, Germany). All microscopic and histological assessments were performed using a blind method to prevent observer bias.

Statistical analysis

All data represent at least three independent experiments and are expressed as mean ± SD. Differences between experimental groups were analysed using Kruskal-Wallis test performed with SPSS software. Individual statistical significance between treatments was assessed using Student's *t*-test. The difference was considered to be statistically significant if the probability value was less than 0.05 ($P < 0.05$).

Table 1 Encapsulation efficiency, drug-loading coefficient and particle size of the micelles obtained

Code	CPT-11 (mg)	PEO-PPO-PEO (mg)	E200-PEG400 (mg)	EE (%)	DL (%)	Particle size (nm)
M1	25	250	0	37.1 ± 2.4	3.37 ± 0.22	20.03 ± 2.01
M2	25	250	75	67.5 ± 2.9	4.82 ± 0.18	22.15 ± 4.12
M3	25	250	150	86.2 ± 3.6	5.07 ± 0.21	22.46 ± 3.41
M4	25	250	300	88.6 ± 4.1	3.85 ± 0.15	58.65 ± 10.50

CPT-11, irinotecan hydrochloride. Encapsulation efficiency, EE; drug-loading coefficient, DL. PEO-PPO-PEO, poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide). Data were the mean ± SD of three determinations.

Results

Characterization of CPT-11-loaded PEO-PPO-PEO micelles

The EE and DL of CPT-11-loaded PEO-PPO-PEO micelles could be adjusted by varying the ratio of E200-PEG400 and PEO_{20}-PPO_{70}-PEO_{20}. By increasing the E200-PEG400 ratio higher EE values were obtained (Table 1). The particle size of the micelles was small and uniform up to E200-PEG400 150 mg. In consideration of the observed EE, DL and particle size, formulation M3 was chosen for the experiments.

The average particle size of CPT-11-loaded PEO-PPO-PEO micelles (formulation M3) was 22.46 ± 3.41 nm, and the polydispersity index (PI) was 0.170. The atomic force microscopic image showed that the obtained micelles were flattened round species, confirming their spherical shape in solution (figure not shown).

Cellular drug accumulation

As previously reported, novobiocin sodium is a specific inhibitor of BCRP.[23] In this study, the drug was utilized to characterize the effect of PEO_{20}-PPO_{70}-PEO_{20} copolymer and PEO-PPO-PEO micelles on the BCRP mediated CPT-11 efflux in MDCKII/BCRP cells. Cells were incubated with CPT-11-loaded micelles, CPT-11 plus PEO_{20}-PPO_{70}-PEO_{20} or Novobiocin sodium for 2 h. Figure 2 shows that MDCKII/BCRP cells with substantial BCRP levels had sevenfold reduced accumulation of CPT-11 compared with MDCKII/wt, and no metabolite was detected. The result suggested that BCRP could mediate the efflux of CPT-11. Novobiocin sodium at 30 μM significantly inhibited BCRP-mediated CPT-11 efflux, and increased the intracellular accumulation of CPT-11 in MDCKII/BCRP by 78.9%. Cells incubated with PEO_{20}-PPO_{70}-PEO_{20} at 3, 5, 10 or 30 μM exhibited increased intracellular drug accumulation by 53.9, 98.2, 115.5 and 106.8%, respectively. PEO_{20}-PPO_{70}-PEO_{20} treatment resulted in higher CPT-11 accumulation, indicating that PEO_{20}-PPO_{70}-PEO_{20} was a potent BCRP inhibitor. Among all the inhibitory treatments, the CPT-11-loaded PEO-PPO-PEO micelles led to the highest drug cellular accumulation, which was significantly higher than those observed with novobiocin or PEO-PPO-PEO treatment ($P < 0.05$).

It was noteworthy that exposure of MDCKII/BCRP cells to PEO_{20}-PPO_{70}-PEO_{20} below the critical micelle concentration (CMC, 4.4 μM at 37°C) resulted in increased accumulation of CPT-11, whereas the intracellular drug accumulation was comparable above the CMC. Therefore, 5, 10 and 30 μM PEO_{20}-PPO_{70}-PEO_{20} were used in the following drug transport experiment.

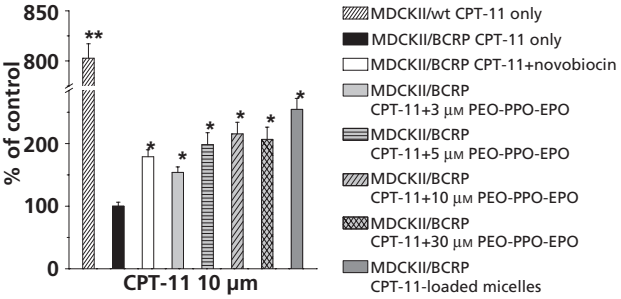

Figure 2 Cellular CPT-11 accumulation in MDCKII/BCRP cells treated with CPT-11 injection, CPT-11 plus PEO-PPO-PEO or novobiocin, or CPT-11-loaded PEO-PPO-PEO micelles. Novobiocin sodium is a specific BCRP inhibitor, used as positive control. Cells were incubated with CPT-11 (irinotecan hydrochloride; 10 μM), CPT-11 (10 μM) plus PEO_{20}-PPO_{70}-PEO_{20} (3, 5, 10 and 30 μM) or novobiocin (30 μM), or CPT-11-loaded PEO-PPO-PEO (poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide)) micelles for 2 h. Each column represents the mean ± SD of three independent experiments. Differences in drug accumulation were evaluated in comparison to MDCKII/BCRP cells incubated with CPT-11 only (used as control); $*P < 0.001$, $**P < 0.0001$.

Drug transepithelial transport

The effects of novobiocin sodium, PEO_{20}-PPO_{70}-PEO_{20}, and PEO-PPO-PEO micelles on the permeability of CPT-11 were investigated in MDCKII/wt and MDCKII/BCRP cells (Table 2). The efflux ratio of CPT-11 permeability (B–A/A–B) in MDCKII/BCRP cells was approximately 6.77-fold greater compared with that in MDCKII/wt, confirming the involvement of BCRP in the efflux of CPT-11.

Novobiocin sodium, a specific inhibitor for BCRP, at the concentration of 30 μM completely reversed the BCRP-mediated efflux of CPT-11 in MDCKII/BCRP cells, with resultant efflux ratio of 11.3. In the presence of PEO_{20}-PPO_{70}-PEO_{20} (5, 10 and 30 μM), the absorptive P_{app} (A–B) of CPT-11 across MDCKII/BCRP cells was significantly increased, while efflux P_{app} (B–A) was significantly decreased. PEO_{20}-PPO_{70}-PEO_{20} at 5, 10 and 30 μM reduced CPT-11 efflux ratios in MDCKII/BCRP cells from 109.5 to 11.4, 10.0, and 11.2, respectively. The efflux ratio was completely reversed, suggesting that PEO_{20}-PPO_{70}-PEO_{20} completely inhibited BCRP. CPT-11-loaded PEO-PPO-PEO micelles led to the lowest efflux ratio, indicating that such micelles exerted the most

Table 2 The apparent permeability coefficient and efflux ratio of irinotecan hydrochloride across MDCKII/wt and MDCKII/BCRP monolayers

Cells	Substrate	P_{app}(×10^{-6} cm/s)		Efflux ratio (P_{app}(B–A)/P_{app}(A–B))
		A–B	B–A	
MDCKII/BCRP	CPT-11	0.57 ± 0.02	62.50 ± 11.85	109.6
	CPT-11+ novobiocin	0.64 ± 0.02*	7.22 ± 0.32**	11.3
	CPT-11 + PEO-PPO-PEO (5 μM)	3.64 ± 0.20***	41.57 ± 1.40*	11.4
	CPT-11 + PEO-PPO-PEO (10 μM)	3.83 ± 0.15***	38.47 ± 0.95*	10.0
	CPT-11 + PEO-PPO-PEO (30 μM)	3.58 ± 0.26***	40.27 ± 0.15*	11.2
	PEO-PPO-PEO micelles	4.11 ± 0.17***	36.21 ± 0.23*	8.8
MDCKII/wt	CPT-11	0.67 ± 0.03**	10.85 ± 0.13**	16.2

Cells were treated with irinotecan hydrochloride (CPT-11)-loaded PEO-PPO-PEO (poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide)) micelles; formulation M3), CPT-11 (10 μM) plus PEO_{20}-PPO_{70}-PEO_{20} (5, 10 and 30 μM), and CPT-11 (10 μM) plus novobiocin (30 μM). MDCKII/BCRP, BCRP overexpressing MDCKII cell line; MDCKII/wt, its corresponding wild type cell line. Micelles were diluted with DMEM, and the final concentrations of CPT-11 and PEO_{20}-PPO_{70}-PEO_{20} were approximately 10 μM. Permeability coefficient, P_{app}. Data represent the mean ± SD of three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, significantly different from control (MDCKII/BCRP CPT-11).

potent inhibitory effect on BCRP. The experimental treatments we used yielded different levels of inhibitory effect on BCRP, specifically PEO-PPO-PEO micelles >PEO_{20}-PPO_{70}-PEO_{20} ≈ novobiocin. These results were consistent with those obtained in the drug cellular accumulation tests.

Biliary excretion of CPT-11 and its metabolites

Previous studies have reported high levels of BCRP expression in the canalicular membrane of hepatocytes in Sprague-Dawley rats.[10] To assess whether the in-vitro inhibitor- CPT-11 interaction via BCRP also occurred *in vivo*, the effect of novobiocin, PEO_{20}-PPO_{70}-PEO_{20}, and PEO-PPO-PEO micelles on hepatobiliary excretion of CPT-11 was investigated. Figure 3 shows the cumulative biliary excretion curves of CPT-11 and its metabolite SN-38 after 15 mg/kg CPT-11, CPT-11 plus novobiocin or PEO_{20}-PPO_{70}-PEO_{20}, or CPT-11-loaded PEO-PPO-PEO micelles were injected intravenously into rats. Compared with CPT-11 injection, biliary excretion of CPT-11 and SN-38 was significantly reduced by PEO-PPO-PEO micelles ($P < 0.05$). A similar tendency was found in the treatments of novobiocin and PEO_{20}-PPO_{70}-PEO_{20}. CPT-11 biliary excretion in 6 h was decreased from 33.3 ± 1.55% to 26.9 ± 1.48% by novobiocin, to 22.0 ± 2.03% by PEO_{20}-PPO_{70}-PEO_{20} and to 16.7 ± 1.48% by CPT-11-loaded PEO-PPO-PEO micelles. Similarly, SN-38 biliary excretion decreased from 7.13 ± 0.87% to 6.04 ± 0.59%, to 5.20 ± 0.69% and to 4.03 ± 0.55%, respectively. The cumulative volumes of biliary excretion exhibited no significant difference among the various treatments (approximately 0.6 ml/h). These results indicated that all the formulations were able to decrease the biliary excretion rate and concentration of CPT-11, although with different efficacy (in descending order, the inhibitory activity in CPT-11 biliary excretion was: PEO-PPO-PEO micelles; PEO_{20}-PPO_{70}-PEO_{20}; novobiocin). It can be inferred that the concentrations of CPT-11 and SN-38 in the intestine were diminished due to the decreased biliary excretion after administration of novobiocin, PEO_{20}-PPO_{70}-PEO_{20}, and PEO-PPO-PEO micelles.

Pharmacokinetics of CPT-11 and its metabolites

The CPT-11 and SN-38 plasma concentration profiles after intravenous administration of CPT-11 injection, CPT-11 plus

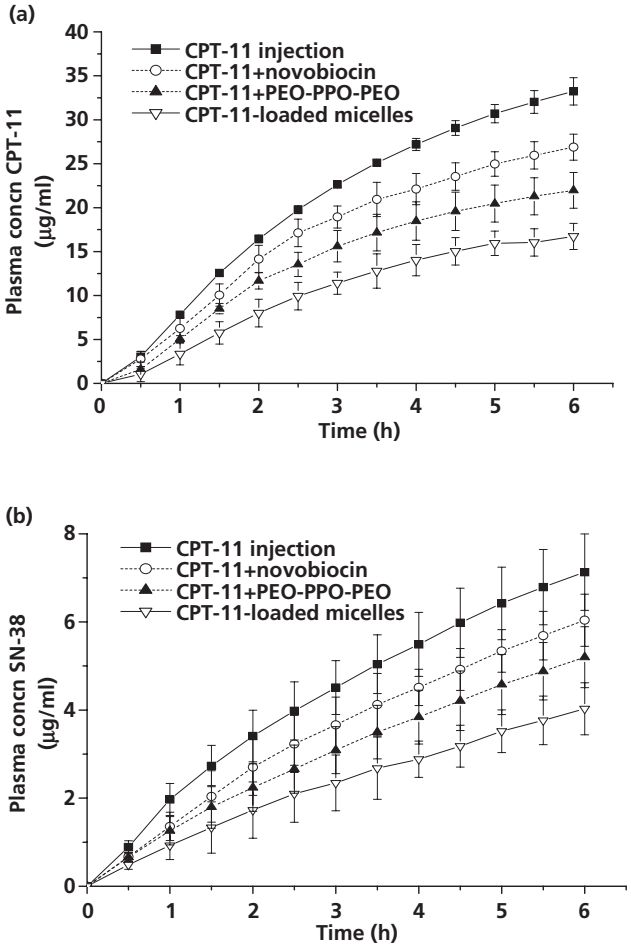

Figure 3 Biliary excretion of CPT-11 and SN-38 after administration of CPT-11 injection, CPT-11 plus novobiocin or PEO_{20}-PPO_{70}-PEO_{20}, or CPT-11-loaded PEO-PPO-PEO micelles in rats. (a) CPT-11 (irinotecan hydrochloride); (b) SN-38 (active metabolite of CPT-11). PEO-PPO-PEO, poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide). The intravenous dose for each treatment was 15 mg/kg. Each data point represents the mean ± SD of data collected from three rats.

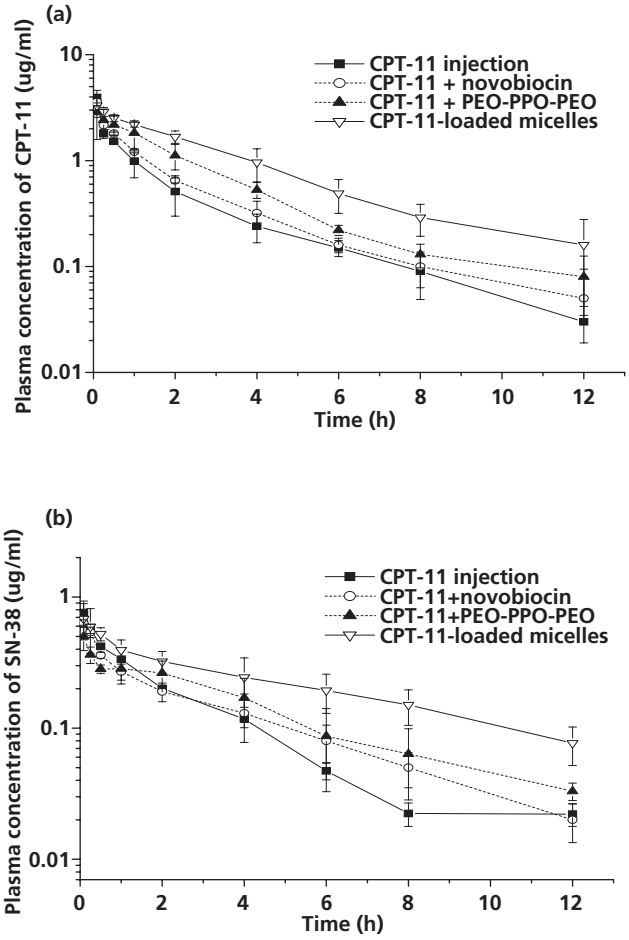

Figure 4 Plasma concentration profiles of CPT-11 and SN-38 after administration of CPT-11 injection, CPT-11 plus novobiocin or PEO_{20}-PPO_{70}-PEO_{20}, or CPT-11-loaded PEO-PPO-PEO micelles in rats. (a) CPT-11 (irinotecan hydrochloride); (b) SN-38 (active metabolite of CPT-11). PEO-PPO-PEO, poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide). The intravenous dose for each treatment was 15 mg/kg. Each data point represents the mean ± SD of measurements from four rats.

novobiocin or PEO_{20}-PPO_{70}-PEO_{20}, and CPT-11-loaded PEO-PPO-PEO micelles are shown in Figure 4, whereas pharmacokinetic parameters are listed in Table 3. The value of area under the curve (AUC) for CPT-11 increased approximately of 2.4-fold after treatment with PEO-PPO-PEO micelles compared with CPT-11 injection ($P < 0.01$). PEO_{20}-PPO_{70}-PEO_{20} and novobiocin also significantly increased the AUC of CPT-11 (7.04 ± 0.46 and 4.95 ± 0.31 vs 4.22 ± 0.27 μg·h/ml $P < 0.05$). Moreover, PEO-PPO-PEO micelles significantly increased the mean residence time (MRT; $P < 0.05$) and decreased clearance (CL; $P < 0.01$) of CPT-11. The changes in pharmacokinetic parameters of SN-38 were similar to those observed for CPT-11: AUC, MRT and half-life ($t^1/_2$) of SN-38 were significantly increased by PEO-PPO-PEO micelles compared with CPT-11 injection.

These results indicated that the increased value for AUC of CPT-11 and SN-38 after novobiocin or PEO_{20}-PPO_{70}-PEO_{20} treatment may have been due to the significant inhibition of the drug biliary excretion. PEO-PPO-PEO micelles were able to increase the systemic circulation time and AUC of CPT-11. This might have been due not only to the inhibitory effect of PEO-PPO-PEO on the drug biliary excretion, but also to the limited opsonization and uptake by the macrophages of the reticuloendothelial system. These results were in good agreement with the observed reduction of drug biliary excretion.

PEO-PPO-PEO micelles and novobiocin reduced CPT-11-induced intestinal damage

Microscopic pathological changes were observed in the intestinal tissues of rats after intravenous administration of CPT-11 injection, CPT-11 plus novobiocin and CPT-11-loaded PEO-PPO-PEO micelles (Figure 5). There were no histological changes in the control rats. Administration of CPT-11 injection resulted in severe damage to the intestinal mucosal membrane. Widespread apoptosis of basal crypt enterocytes, stubby villi or patches without villi, dilated crypt lumina and inflammatory cellular infiltration were observed in the ileum. Damage in the cecal mucosal membrane was characterized by destruction of normal mucosal architecture, degeneration of crypts and oedema in the submucosa. Widely disseminated enterocyte apoptosis occurred in the colon.

Intestinal toxicity was minimized in rats that received CPT-11 plus Novobiocin sodium or CPT-11-loaded micelles. Novobiocin sodium treatment significantly increased the villus height in the ileum, and preserved the mucosal morphology in the colon. However, a minor destruction of the mucosal architecture could still be observed in the ileum and cecum. PEO-PPO-PEO micelles significantly reduced the histological alteration observed in the ileum, cecum and colon. The intestinal mucosal morphology observed in rats treated with micelles, including villus height and crypt structure, was almost identical to that observed in the control group.

Discussion

It has been reported that not only some inhibitors, but several common excipients such as Pluronic P85, Cremophor EL and Tween 20, can modulate the activity of efflux transporters.[24] PEO-PPO-PEO, amphiphilic synthetic polymers, could serve as nanomaterial inert carriers as well as a biological response modifier. PEO-PPO-PEO micelles can increase solubility, improve bioavailability of drugs and release them at the target sites. In addition, they can release individual block copolymer molecules, which are shown to inhibit efflux transporters, and change drug behaviour *in vivo*.

The inhibitory effect of PEO-PPO-PEO micelles on BCRP

CPT-11, a substrate of BCRP, can be intercepted by this efflux pump in the cellular membrane, and flipped to the extracellular domain. We evaluated the effect of PEO_{20}-PPO_{70}-PEO_{20} and PEO-PPO-PEO micelles on BCRP-mediated cellular efflux of CPT-11 in MDCKII/wt and MDCKII/BCRP cells (Figure 2). Novobiocin sodium, a specific inhibitor of BCRP, was used as a positive control.[23] The results suggested that BCRP played a critical role in CPT-11 efflux across MDCKII cells. PEO_{20}-PPO_{70}-PEO_{20} significantly increased the uptake

Table 3 Pharmacokinetic parameters of CPT-11 and SN-38 after intravenous administration into rats of CPT-11 injection, CPT-11 plus novobiocin or PEO_{20}-PPO_{70}-PEO_{20} or CPT-11-loaded PEO-PPO-PEO micelles with a single dose of CPT-11

Metabolites	Parameters	CPT-11 injection	CPT-11 + novobiocin	CPT-11 + PEO-PPO-PEO	CPT-11-loaded micelles
CPT-11	$AUC_{0\text{-}12h}$ (μg·h/ml)	4.22 ± 0.27	4.95 ± 0.31*	7.04 ± 0.46*	10.36 ± 0.94**
	C_{max} (μg /ml)	3.95 ± 0.65	3.58 ± 0.29	2.60 ± 1.28	3.32 ± 0.67
	MRT (h)	2.20 ± 0.26	2.21 ± 0.12	2.50 ± 0.18	3.06 ± 0.33*
	$t^1/_2$ (h)	2.04 ± 0.28	2.25 ± 0.19	2.36 ± 0.17	2.47 ± 0.35
	CL (1/h/ml)	3.47 ± 0.31	3.04 ± 0.27	2.11 ± 0.22*	1.41 ± 0.21**
SN-38	$AUC_{0\text{-}12h}$ (μg·h/ml)	1.40 ± 0.06	1.45 ± 0.09	1.57 ± 0.21	2.69 ± 0.36**
	C_{max} (μg /ml)	0.76 ± 0.17	0.63 ± 0.04	0.50 ± 0.11	0.68 ± 0.21
	MRT (h)	2.93 ± 0.20	3.13 ± 0.22	3.32 ± 0.31	4.13 ± 0.27**
	$t^1/_2$ (h)	3.09 ± 0.11	3.10 ± 0.08	3.21 ± 0.12	4.45 ± 0.20**
	CL (1/h/ml)	10.60 ± 0.74	9.74 ± 0.95	8.89 ± 1.01	5.40 ± 0.98**

CPT-11, irinotecan hydrochloride; SN-38, active metabolite of CPT-11. Novobiocin sodium 50 mg/kg; PEO_{20}-PPO_{70}-PEO_{20} 75 mg/kg; single dose CPT-11 15 mg/kg. PEO-PPO-PEO, poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide). AUC, area under the curve; C_{max}, maximum plasma concentration; MRT, mean residence time; $t^1/_2$, half-life; CL, clearance. Data represent the mean ± SD of measurements from four rats. *$P < 0.05$, **$P < 0.01$ vs CPT-11 injection.

of CPT-11 in MDCKII/BCRP cells, confirming its inhibitory effect on BCRP. It is worthy to note that CPT-11 accumulation in MDCKII/BCRP cells depended on the concentration of PEO_{20}-PPO_{70}-PEO_{20}. In fact, exposure of MDCKII/BCRP cells to CPT-11 with PEO_{20}-PPO_{70}-PEO_{20} below the CMC resulted in increased drug accumulation. In contrast, above the CMC, the copolymer added in the system was consumed for the formation of the micelles, thus the CPT-11 uptake revealed a tendency towards levelling off. This result was similar to the previously reported effect of Pluronic on P-gp-mediated R123 efflux in multiple drug resistant cells.[25] Therefore, the CMC provided the 'cut-off point' for the CPT-11 accumulation in MDCKII/BCRP cells.

Among various treatments, PEO-PPO-PEO micelles were most effective for the enhancement of CPT-11 uptake by MDCKII/BCRP cells. Two factors may have contributed to this phenomenon. Factor one, the encapsulated CPT-11 may have been delivered into the cells by the micelles via an endocytic pathway, which could bypass BCRP mediated efflux. Factor two, PEO-PPO-PEO and CPT-11 could be released from the carrier at the same time outside the cell, and the high local concentration domains of CPT-11 and PEO-PPO-PEO automatically overlapped with each other. As a result, BCRP transporters that were exposed to high local CPT-11 concentration would be strongly inhibited by the PEO-PPO-PEO molecules that were in close proximity. This effect might have created windows in the cell membrane that could allow CPT-11 to enter into the cytoplasm with minimal BCRP-mediated efflux.

In the study of CPT-11 transport across BCRP overexpressing cells, MDCKII cells could form a confluent monolayer with tight junctions and could be used as a surrogate for physiological barriers to examine the transport of drug *in vitro*. BCRP has been localized on the apical side of plasma membranes and is known to preferentially transport its substrates via the B–A direction.[26] CPT-11 was reported to be the substrate of BCRP, and its B–A transport was significantly higher than the A–B transport across the MDCKII/BCRP cell monolayer (Table 2). As shown in Table 2, novobiocin, PEO_{20}-PPO_{70}-PEO_{20}, and PEO-PPO-PEO micelles significantly increased A–B directed transport and decreased B–A directed transport of CPT-11 in MDCKII/BCRP cells. As a result, the efflux ratios for these groups were approximately 90% lower than those treated with CPT-11 alone. The inhibitory effects of the various treatments on BCRP were in agreement with the results of drug cellular accumulation. The exact mechanism by which PEO-PPO-PEO inhibited efflux transporters is unknown. It has been reported that PEO-PPO-PEO significantly reduced the intracellular ATP level by inhibiting respiration in mitochondria, and this might result in the inhibition of efflux transporters.[27] Meanwhile, the integrity of cell membrane was not affected in the presence of Pluronic.[28]

BCRP role in CPT-11 behaviour *in vivo* induced by PEO-PPO-PEO micelles

Previous studies have indicated that CPT-11 may interact with some drugs, causing high bioavailability or low biliary excretion.[29,30] The mechanisms responsible for these reported interactions have been mostly related with the modulation of drug–enzymes and/or P-gp. To date, the biliary excretion of CPT-11 mediated by other major efflux drug transporters has not been studied thoroughly. Therefore, we decided to study the potential involvement of BCRP mediated biliary CPT-11 excretion *in vivo*. BCRP expressed in the liver plays an important role in the biliary excretion of drugs and metabolites.[13] Both CPT-11 and SN-38 were reported to be substrates of BCRP.[31] To confirm the participation of BCRP in CTP-11 excretion, novobiocin sodium, a specific inhibitor of BCRP, was coadministered with CPT-11 and the biliary excretion of CPT-11 and SN-38 was investigated. Our results showed that novobiocin reduced the biliary excretion of CPT-11 and SN-38 (Figure 3). This may have been due to the inhibition of the expulsion of CPT-11 and SN-38 across the apical membranes via BCRP localized in the liver canalicular epithelial cells. Therefore, BCRP was considered to be involved in the biliary excretion of CPT-11 and SN-38.

As shown in Figure 3, PEO_{20}-PPO_{70}-PEO_{20} inhibited CPT-11 biliary excretion more effectively than novobiocin. CPT-11 is not a unique substrate for BCRP, but is also a poor

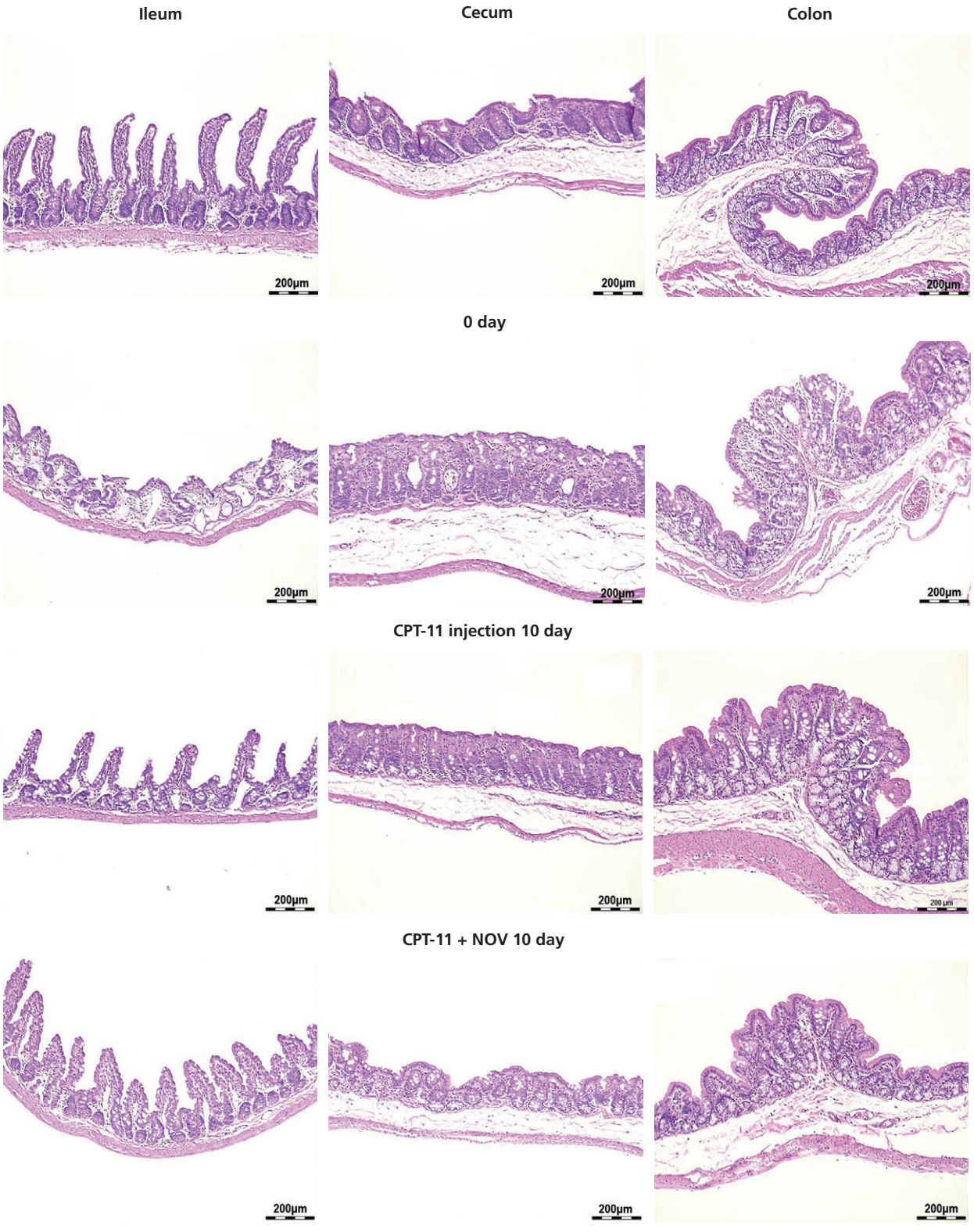

Figure 5 Micrographs of rat intestinal tissues showing histological damage after administration of CPT-11 injection, CPT-11 plus novobiocin or CPT-11-loaded PEO-PPO-PEO micelles. Micrographs of the ileum, cecum and colon were taken at day 0 and day 10 to examine any damage resulting from the different intravenous treatments. CPT-11, irinotecan hydrochloride; NOV, novobiocin sodium; PEO-PPO-PEO, poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide). Magnification ×200.

P-gp and MRP2 substrate.[31] PEO-PPO-PEO copolymers have been reported to exhibit an inhibitory effect on P-gp.[25] Therefore, we could not exclude the possibility that the reduced biliary excretion of CPT-11 by PEO_{20}-PPO_{70}-PEO_{20} was due to the inhibition of other efflux transporters in addition to BCRP. Decrease in biliary excretion of CPT-11 and SN-38 was reported to be related with the inhibition of P-gp.[25] Thus, PEO_{20}-PPO_{70}-PEO_{20} might have reduced the biliary excretion of CPT-11 through inhibition of drug efflux transporters including BCRP, P-gp and MRP2.

To investigate further the interactions of novobiocin and PEO_{20}-PPO_{70}-PEO_{20} with CPT-11 *in vivo*, pharmacokinetic studies were performed in rats. After intravenous administration, PEO-PPO-PEO micelles significantly increased the value of the AUC and decreased the CL of CPT-11 (Table 3). Novobiocin had a similar effect on the AUC value of CPT-11, and it also showed a tendency to decrease CL. The increased AUC could be attributed to decreased systemic elimination, and it was consistent with the inhibitory effect of PEO_{20}-PPO_{70}-PEO_{20} and novobiocin on CPT-11 biliary elimination.

PEO-PPO-PEO micelles were most effective in the inhibition of biliary excretion, increasing plasma concentration and improving the AUC value of CPT-11 / SN-38 (Figures 3 and 4). This was probably due to the following two factors: firstly, the hydrophilic shell of PEO could prevent the recognition of the micelles by the reticuloendothelial system and prolong the blood circulation time of the drug, thereby decreasing the clearance rate of CPT-11 and SN-38; secondly, high local concentration of PEO-PPO-PEO associated with CPT-11 could exert an inhibitory effect on drug efflux transporters.

In this study, the PEO-PPO-PEO micelles obtained reduced the biliary excretion and increased the AUC values of CPT-11 and SN-38. PEO-PPO-PEO is commonly used in human pharmaceutical formulations. Its inhibitory effect on BCRP has been reported to be transient and reversible.[32] Obviously, for clinical applications, a transient effect on BCRP is preferable to a persistent one. BCRP mediates the secretion of several carcinogens and dietary toxins, and so a transient effect could minimize any serious adverse effects that might be caused by prolonged BCRP inhibition. Therefore, the use of PEO-PPO-PEO micelles as a drug carrier and transporter inhibitor may result in clinically pharmacotherapeutic benefits. The PEO-PPO-PEO micelles represent a promising carrier for CPT-11 because they could increase its anticancer therapeutic effect while decreasing its intestinal toxicity.

The mechanism of CPT-11-induced intestinal damage

The intestinal damage was evaluated by histological analysis. It revealed that treatment with novobiocin or PEO-PPO-PEO micelles could reduce CPT-11-induced intestinal mucous membrane damage. It has been proposed that the severe gastrointestinal toxicity induced by CPT-11 results from exposure of intestinal tissues to CPT-11 and SN-38. CPT-11 is metabolized in the liver by carboxylesterase to SN-38, which is conjugated to SN-38G and excreted into the intestine via bile. SN-38G excreted into the gut is hydrolysed to SN-38 by enterobacterial β-glucuronidase, and subsequently damages the gut. The mechanisms for CPT-11-induced intestinal mucosal membrane damage appeared to be associated with intestinal exposure to these drugs and consequent induction of epithelial apoptosis, characterized by the generation of DNA fragments through the action of endogenous endonucleases. The homeostatic balance between proliferation and apoptosis is essential for the intestinal epithelium to function as a physiological and structural barrier. Disruption of this balance leads to villus atrophy and loss of normal absorptive function. Cytotoxic drugs (e.g. CPT-11) can disrupt this mucosal barrier by increasing epithelial apoptosis, and cause severe intestinal mucosal membrane damage. In addition, CPT-11 has several pharmacological effects that can cause acute intestinal damage. In fact, it induces a transient increase in prostaglandin E_2 (PGE_2) in the intestinal tissue.[33] PGE_2 is secreted by the mucosa and smooth muscle of the small intestine, and induces diarrhoea by stimulating colonic secretion and hyperperistalsis of the gut.[34] Moreover, PGE_2 inhibits Na^+,K^+-ATPase, thereby affecting the absorption of electrolytes.[35] CPT-11 induces Cl^- secretion in the subepithelial tissue by stimulating the production of eicosanoid, such as thromboxane A_2.[36] These effects of CPT-11 may not only exacerbate the diarrhoeal symptoms and the impairment of cecal tissue, but alter the intestinal luminal environment and induce colonization by indigenous microflora and an over growth of pathogenic bacteria. Novobiocin and PEO-PPO-PEO micelles could inhibit BCRP function and decrease the biliary excretion of CPT-11 and SN-38, consequently diminishing epithelial impairment of the intestinal mucosal membrane. This was consistent with the results regarding the biliary excretion.

Conclusions

PEO-PPO-PEO micelles have been developed to encapsulate CPT-11. Various strategies for administering CPT-11 (PEO-PPO-PEO micelles and coadministration with novobiocin sodium or PEO_{20}-PPO_{70}-PEO_{20}) were systematically compared. It was found that among the investigated approaches, treatment with PEO-PPO-PEO micelles resulted in the highest uptake of CPT-11 by BCRP overexpressing MDCK cells and the lowest CPT-11 biliary excretion. Moreover, such treatment exhibited the best efficiency in ameliorating intestinal toxicity and increasing CPT-11 circulation time in the blood. PEO_{20}-PPO_{70}-PEO_{20} in micelles worked both as nanocarrier and BCRP inhibitor. The micelles could release copolymer molecules and locally inhibit the BCRP-mediated CPT-11 efflux in the canalicular membrane of hepatocytes, thus decreasing intestinal toxicity. In addition, the hydrophilic blocks of PEO-PPO-PEO could avoid uptake by the reticuloendothelial system, prolonging drug circulation time in the blood and consequently increasing its systemic exposure. These findings indicate that PEO-PPO-PEO micelles represent a promising drug carrier that could be exploited to reduce intestinal toxicity and increase antitumour therapeutic effect of CPT-11. Our results have provided proof for a potential involvement of BCRP in the pharmacokinetics and intestinal toxicity of CPT-11.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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