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Supporting information:

Loading docetaxel in β-cyclodextrin-based micelles for enhanced oral chemotherapy through inhibition of P-glycoprotein mediated efflux transport

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Experimental Section

1.1. Synthesis and Characterization of PELC Copolymer

A predetermined amount of mPEG (0.5 mmol), LA (0.014 mol) and 0.5 wt% $Sn(Oct)_2$ reacted at 130 °C for 24 h. The PEL copolymer was precipitated in cold diethyl ether twice. The overall yield was more than 85%. Then PEL (0.1 mmol), succinic anhydride (0.12 mmol), DMAP (0.12 mmol), and triethylamine (0.12 mmol) were dissolved in anhydrous 1,4-dioxane and stirred for 24 h at room temperature. Then the product was precipitated twice in ethyl ether and vacuum drying overnight. Then, β-CD (0.1 mmol), CDI (0.4 mmol), and TEA (0.4 mmol) were dissolved in dry dimethyl sulphoxide (DMSO) and reacted at room temperature for 3 h. Afterward, PEL-COOH (0.12 mmol) was added. After reacting overnight, the product PELC was precipitated twice in cold ethyl ether and further purified by dissolving in acetone, dialyzing against water for 2 d and lyophilization.

^{1}H-NMR spectra were recorded on a Varian 500-MHz NMR spectrometer (Varian Inc., Palo Alto, CA, USA) using DMSO-d6 and D_2O as solvent, respectively. The molecular weight and distribution (PDI=M_w/M_n) of PELC copolymer was determined by gel permeation chromatography equipped with a Waters 515 HPLC pump, a Waters Styrage™ HT3 GPC column (300nm in length and 7.8 mm in diameter), and a Waters 2410 refractive index detector. The critical micelle concentration (CMC) of the polymer was determined to prove the potential of micelle formation by fluorescence measurements using pyrene as a probe. The size of the micelles was measured by dynamic light scattering (DLS, Zetasizer Nano S90, Malvern, UK).

1.2. Preparation and Characterization of DTX-Loaded Micelles

The size of the drug loaded micelles was measured by DLS. The morphological examination of the micelles was performed using a transmission electron microscope (TEM, JEM-1230, JEOL Ltd. Tokyo, Japan). The amount of entrapped DTX in micelles was determined by HPLC (Shimadzu, Japan) analysis at a wavelength of 230nm. The HPLC system was equipped with a UV-Vis detector (SPD-M20A) and a Diamonsil™C18 column (150*4.6 mm, 5 μm), acetonitrile/water (55:45, v/v) as the

mobile phase at 1.0 mL/min.

1.3. Transport Studies across the MDCK and MDCK-MDR1 Cell Monolayer

The integrity of cell monolayers was characterized by net transepithelial electrical resistance (TEER) and the permeability *was* characterized *by* lucifer yellow (Ex/Em 485/525) which transport*s* via the paracellular route. Cell monolayers were used in transport assay when TEER exceeded 250 Ω·cm^{2}and apparent permeability coefficients (Papp) of lucifer yellow was below *1*×10^{-6} cm/s. The TEER value was measured by a Millicell-ERS voltohmmeter (Millipore Co., USA). The intrinsic resistance of the system (insert alone) was subtracted from the total resistance (cell monolayer plus insert) to yield the TEER. After the transport experiment, the integrity of the monolayers was assessed by means of TEER measurements.

For the transport experiments, MDCK or MDCK-MDR1 cells were seeded onto Transwell filters at a density of 2.5×10^5 cells per well and were allowed to grow and differentiate for 4 d. Prior to the transport experiment, culture media was removed from the mature monolayers and the monolayers were rinsed twice with 37 °C HBSS. After 15 min incubation of both sides of the monolayers with HBSS (0.5 mL to apical side and 1.0 mL to basolateral side), 0.5 mL of HBSS solution containing 60 μg/mL DTX of various groups including free DTX, PELC/DTX micelles, PEL/DTX micelles, free DTX with 100 μM verapamil, free DTX with 1.2 mg/mL PELC or free R123 was applied to the apical side followed by addition of 1.0 mL of HBSS solution to the basolateral side for absorptive (AP to BL) transport, while secretory (BL to AP) transport was evaluated adding 1.0 mL of drug solution in basolateral side and 0.5 mL of HBSS in apical side. Cells were incubated in a 37 °C or 4 °C shaking incubator. 200 μL of aliquots were taken from the basolateral side (to study absorptive transport) and the apical side (to study secretory transport) at 20, 40, 60, 80, 100 and 120 min time intervals. The same volume of fresh prewarmed HBSS was added to keep the volume constant. The content of DTX or R123 was detected by HPLC or Multifunctional microplate reader SpectraMax M5 (Molecular Devices, USA) at Ex/Em 480/535.

1.4. In Situ Single Pass Intestinal Perfusion (SPIP)

Male Sprague-Dawleyrats (weighting 200-250 g) fasted for 12 h (free access to water) were anesthetized with chloralhydrate (350 mg/kg) by intraperitoneal injection. A midline longitudinal incision was made on the abdomen. Two PVC tubings were inserted at the proximal and distal ends of the located 15cm jejunal segments. The inlet tube was connected to the peristaltic pump (BT100-2J, Baoding Longer Precision Pump Ltd. China) to offer a constant flow rate of 1 mL/min with perfusion solution for 20 min until the steady state (as the inlet over outlet concentrations ratio of phenol red approaches 1) had been reached. Thereafter the flow rate turned to 0.2 mL/min and the outflow samples were collected at 15 min intervals up to 2 h. The levels of phenol red were tested by UV spectrometer at 558 nm and the concentrations of DTX were tested by HPLC. At the ending of experiment, the animals were sacrificed. The length and radius of the perfused jejunal segment were measured.

1.5. Pharmacokinetic Studies

The mice were fasted 12 h before experiment with free access to water and were divided randomly into three sets: group 1, free DTX solution (2 mg/kg) via intravenous injection (i.v.); group 2, oral gavage with free DTX-water suspension (20 mg/kg); group 3, oral gavage with PEL/DTX (20 mg/kg); group 4, oral gavage with PELC/DTX (20 mg/kg). At predetermined time points, 0.6 mL of blood samples were collected into EDTA-2K tubes and centrifuged at 8000 rpm for 10 min to obtain plasma. 0.2 mL of acetonitrile was added to 0.2 mL of plasma and the resulting mixture vortexed vigorously for 2 min and centrifuged at 15000 rpm for 15 min. Then the supernatant was obtained to detect DTX concentration. The pharmacokinetic parameters of plasma concentration-time data were analyzed by statistical moment theory using DAS 2.0.

1.6. *In Vivo* Anti-Tumor Activity and Toxicity Study

The *in vivo* inhibitory efficacy of free DTX, PELC/DTX micelle and PELC/DTX micelle via different administration was evaluated against S180 solid tumor. After S180 cells being subcutaneously implanted into the armpits of the mice, the tumor bearing mice were randomly divided into six groups (5 mice per group): group 1, control (normal saline); group 2, intravenous injection with free DTX (10 mg/kg); group 3, oral gavage with free DTX (20 mg/kg); group 4, oral gavage with free DTX

(30 mg/kg); group 5, oral gavage with PELC/DTX micelle (20 mg/kg); group 6, oral gavage with PELC/DTX micelle (30 mg/kg); group 7, oral gavage with PEL/DTX micelle (30 mg/kg). The dose schedule started when the tumor volume was about 100-200mm^3 and treatments were given every other day for 4 times.

On the day 15, the animals were sacrificed, the blood and tumor mass were harvested. The tumor mass was then weighted, photographed, and processed for histopathological examination.

Histopathological evaluation was performed to determine the toxicity issues pertaining to the oral delivery of free DTX , PEL/DTX micelle and PELC/DTX micelle. Briefly, the xenograft tumors, stomachs and intestines of various treatment groups were fixed with 4 % formaldehyde and embedded in paraffin. Each section was cut into 4 μm, processed for routine hematoxylin and eosin (H&E) staining, and then visualized under microscope. Blood samples were collected from the orbit and samples were measured by ADVIA 2120 hematology analyzer (Siemens Healthcare Diagnostics, Deerfield, IL) to obtain hematological parameters including number counts of neutrophils (NEUT), white blood cells (WBC), and red blood cells (RBC).

Supplementary Tables and Figures

Table S1 Transport and permeability of PEL/DTX across MDCK-MDR1 cells at 37 °C.

Micelle	Temperature	Papp (10^{-6}cm/s)		ER
PEL/DTX	37 °C	A-B	0.580±0.0850	2.072
		B-A	1.202±0.048	

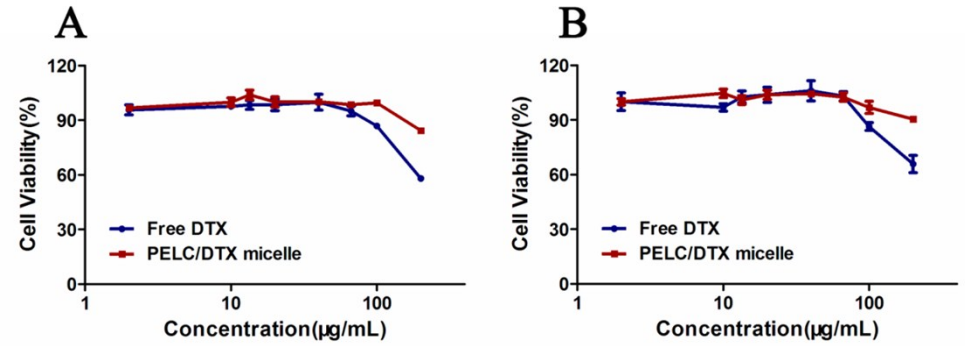

Fig. S1. Cytotoxicity of free DTX and PELC/DTX micelles to (A) MDCK cells and (B) MDCK-MDR1 cells with 2~ 200 μg/mL DTX.