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Preparation and evaluation *in vitro* and *in vivo* of docetaxel loaded mixed micelles for oral administration

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ABSTRACT

A mixed micelle that comprised of monomethylol poly(ethylene glycol)-poly(D,L-lactic acid) (MPP), D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) and stearic acid grafted chitosan oligosaccharide(CSO-SA) copolymers was developed to enhance the oral absorption of docetaxel (DTX). DTX-loaded MPP/TPGS/CSO-SA mixed polymeric micelles (MPMs) were prepared with thin film hydration method and characterized in terms of morphology, size, zeta potential, encapsulation efficiency, critical micellization concentration, and *in vitro* stability in media modeling physiological conditions. The *in vitro* release of docetaxel from the mixed micelles was studied with dialysis method. The oral bioavailability studies were conducted in rats and the pharmacokinetic parameters were evaluated. The results showed that DTX-loaded MPP/TPGS/CSO-SA MPMs had a mean diameter of 34.96 nm and exhibited spherical shape under transmission electron microscopy. The drug loading of DTX in the mixed micelles was 19.15%. The critical micellization concentration of MPP/TPGS/CSO-SA copolymer was 2.11×10^{-5} M, and the size of mixed micelles in gastric fluid (pH 1.6) for 2 h and simulated intestinal fluid (pH 6.5) for 6 h showed no significant change. The *in vitro* release study showed that DTX-loaded MPP/TPGS/CSO-SA MPMs exhibited slower release characteristics compared to DTX solution. The oral bioavailability of the DTX-loaded MPP/TPGS/CSO-SA MPMs was increased by 2.52 times compared to that of DTX solution. The current results encourage further development of DTX mixed polymeric micelles as the oral drug delivery system.

1. Introduction

Docetaxel (DTX) is a potent anticancer drug used to treat various cancers including metastatic and the androgen-independent prostate cancer, breast cancer, and advanced non-small cell lung cancer [1,2]. DTX inhibits cell growth by binding to microtubules, stabilizing them, and preventing their depolymerization. However, the clinical application of DTX is limited by the poor aqueous solubility, low bioavailability and high toxicity. The currently marketed form of DTX (Taxotere®) for intravenous infusion is formulated utilizing Tween 80 and ethanol. Due to hemolysis caused by Tween 80, patients were often subjected to hypersensitivity after administration. To avoid these disadvantages, enhance the patient's convenience, and facilitate the use of more chronic treatment regimens, many studies have been directed toward developing new oral formulations of DTX [3–5].

Oral administration of anticancer agents, such as DTX, represents the easiest and the most convenient route of drug delivery. Moreover, it facilitates a prolonged exposure to the cytotoxic agent and could ease the use of more chronic regimens. Therefore, the enhancement of oral bioavailability of emerging cytotoxic agents is gaining the increasing attention for successful development of oral modes for cancer and leukemia treatment. Unfortunately, the bioavailability of DTX after oral administration is very poor. Several investigators reported that the poor bioavailability of DTX was resulted from the membrane transporter P-glycoprotein (ABCB1) [6,7] as well as poor solubility and permeability. To overcome these drawbacks, several approaches have been investigated, such as co-administration with a P-glycoprotein inhibitor [8], solubilization in self-emulsifying or self-micro-emulsifying drug delivery systems [9], formation of inclusion complexes with cyclodextrins [10], and lecithin nanoparticles [11].

Polymeric micelles (PMs) have a core-shell structure, and the inner core is the hydrophobic part of the polymer, which can incorporate poorly soluble drugs, while the outer shell or the corona

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of the hydrophilic part of the polymer protects the drug from inactivation in biological environment such as the gastrointestinal tract [12]. Due to their small particle size (<200 nm), PMs could be absorbed in their intact form *via* endocytosis which transported macromolecules through enterocyte cell membrane [13,14]. Moreover, PMs possess high loading capacity for poorly water soluble drugs, significantly lower critical micelle concentration (CMC) values and longer lifetime (indicating greater thermodynamic stability) than those of low-molecular weight surfactants [15]. All these issues related to PMs make them become ideal carriers of anticancer drugs for oral delivery. More recently, a large number of studies on mixed polymeric micelles (MPMs) have appeared because the prominent advantages for different types of copolymers concentrated in a single polymeric micellar system. Di/multifunctional PMs can be realized by preparing mixed micelles. The loading content and stability of drug in mixed micelles can be improved greatly with different kinds of polymers compared with single polymer micelles. The release and function of micelles can be modified to be desirable by forming MPMs. Therefore, MPMs would be an ideal formulation that can solubilize DTX efficiently and solve some of the aforementioned problems.

The amphiphilic block copolymer methoxy poly(ethylene glycol)-poly(lactide) (mPEG-PLA, MPP) was often used because of its excellent micelle formation capacity, drug loading capability and release behavior [16,17]. D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) is a PEGylated-vitamin E, which has greatly improved the pharmaceutical properties of vitamin E and thus has been widely applied in the food and drug industry. It has been found that TPGS can enhance the solubility [18], inhibit P-gp-mediated multidrug resistance [19], and increase the oral bioavailability of anticancer drugs [20]. Chitosan (CS) is a cationic polysaccharide with good safety and biocompatibility, which can open the tight junctions [21] and improve the oral uptake of hydrophilic drugs such as peptides. Despite its favorable biological properties, CS is rarely used in oral administration of drugs due to its low solubility under the physiological conditions and limited capacity for controlling the release of drugs. Chemical modification of CS was a feasible way to overcome those disadvantages, making it more suitable as oral delivery vector. Stearic acid grafted chitosan oligosaccharide (CSO-SA) is a kind of hydrophobic modification of CS with low molecular weight [22]. The CSO-SA could form micelles in the aqueous medium, and the CSO-SA micelles could be rapidly internalized into cancer cells [23]. Possessing the same merits as CS, such as biocompatibility, biodegradability and muco-adhesivity, CSO-SA could also be used as a promising oral drug carrier.

In the present study, the DTX loaded mixed micelles composed of MPP, TPGS and CSO-SA were prepared to increase the aqueous solubility and oral absorption of DTX. The physicochemical characteristics of DTX-loaded MPMs such as micro-morphology, size, zeta potential, critical micelle concentration, the *in vitro* stability in modeling physiological conditions of gastrointestinal tract and *in vitro* release were investigated. In addition, the oral bioavailability of DTX-loaded MPMs in rat was also evaluated.

2. Materials and methods

2.1. Materials and animals

DTX was purchased from Baoji Guokang Biotechnology Co., Ltd. (Shanxi, China). $mPEG_{2000}$-PLA_{2000} was obtained from Jinan Daigang Biotechnology Co., Ltd. (Shandong, China). TPGS was purchased from Eastman Co. (USA). Chitosan (M_w = 5 kDa, 85% deacetylated degree) was supplied by Haidebei Marine Biological Engineering Co., Ltd. (Shandong, China). Stearic acid was purchased from Tianjin Kemeng Chemical Industry Co., Ltd. (Tianjin, China). 1-Ethyl-3-(3-dimethylami-nopropyl) carbodiimide (EDC) was purchased from Shanghai Aladdin Co., Ltd. (China).

Male Wistar rats (250 ± 20 g) were supplied by Laboratory Animals Center of Shandong University, Jinan, China. The animals were used following the guidelines of the Ethical Committee for Animal experiments of Shandong University. The animals were acclimatized at a temperature of 25 ± 2 °C and a relative humidity of 70 ± 5% under natural light/dark conditions for at least 24 h before dosing.

2.2. Synthesis and characterization of CSO-SA.

The CSO-SA copolymer was synthesized *via* the reaction of carboxyl groups of SA and amine groups of CSO in the presence of EDC, as described in previous report [24]. Briefly, 0.4460 g of CSO was dissolved in 20 mL of distilled (DI) water and heated up to 60 °C. SA and EDC with the molar ratio of 1–10 were dissolved in 10 mL of an ethanol/acetone mixture (ethanol/acetone = 2/5, *v*/*v*). After stirring for 1 h at 400 rpm, 60 °C, the solution was added into CSO aqueous solution, followed by stirring for another 24 h. Then, the reaction solution was dialyzed against DI water using a dialysis membrane with MWCO of 3.5 kDa (Solarbio, China) for two days, and then the reaction solution was lyophilized. Then the lyophilized product was further purified with ethanol to remove the byproduct. Finally, the ethanol was evaporated to obtain the CSO-SA product.

2.3. Preparation of micelles

MPP/TPGS/CSO-SA MPMs were prepared by thin film hydration method as described earlier [25]. Briefly, CSO-SA (10 mg) was dissolved in 3 mL water. Acetonitrile solution of MPP and TPGS with different weight ratio (the total amount of MPP and TPGS was 30 mg) was evaporated by rotary evaporation at 37 °C to obtain a solid polymer matrix. Residual acetonitrile remaining in the film was removed under vacuum overnight at room temperature. Then, the film was dispersed with CSO-SA solution at 60 °C and vortexed for 5 min, the mixture was then centrifuged to obtain a clear micellar solution. DTX-loaded MPP/TPGS/CSO-SA MPMs were also prepared as described above, except for the addition of DTX in acetonitrile.

DTX-loaded or blank MPP/TPGS MPMs were prepared by replacing the CSO-SA solution with water.

2.4. Characterization of the mixed micelles

Transmission electron microscope (TEM, JEM-1200EX, JEOL, Tokyo, Japan) was performed to evaluate the surface morphology of micelles after negative staining with phosphotungstic acid solution (2%, *w*/*v*). The mean particle size and zeta potential of the micelles were determined by dynamic light scattering (DLS, DELSA^{TM}NANO particle size and zeta potential analyzer, Beckman Coulter Inc., USA). All measurements were performed at 25 °C. Experimental values were calculated from the measurements performed at least in triplicate.

2.5. Determination of docetaxel content in mixed micelles

To determine drug loading content (DL), encapsulation efficiency (EE) and precipitated drug percentage (PD) of micelles, the DTX-loaded MPMs was dissolved with acetonitrile properly and vortexed to get a clear solution, after which the concentration of DTX was measured by high performance liquid chromatography (HPLC). The DL, EE and PD were calculated using the following equations:

$$\mathrm{DL}\% = \frac{\text{weight of the drug in micelles}}{\text{weight of the feeding polymer and drug}} \times 100\% \quad (1)$$