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Azo polymeric micelles designed for colon-targeted dimethyl fumarate delivery for colon cancer therapy

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ABSTRACT

Colon-targeted drug delivery and circumventing drug resistance are extremely important for colon cancer chemotherapy. Our previous work found that dimethyl fumarate (DMF), the approved drug by the FDA for the treatment of multiple sclerosis, exhibited anti-tumor activity on colon cancer cells. Based on the pharmacological properties of DMF and azo bond in olsalazine chemical structure, we designed azo polymeric micelles for colon-targeted dimethyl fumarate delivery for colon cancer therapy. We synthesized the star-shape amphiphilic polymer with azo bond and fabricated the DMF-loaded azo polymeric micelles. The four-arm polymer star-PCL-azo-mPEG (sPCEG-azo) (constituted by star-shape PCL (polycaprolactone) and mPEG (methoxypolyethylene glycols)-olsalazine) showed self-assembly ability. The average diameter and polydispersity index of the DMF-loaded sPCEG-azo polymeric micelles were 153.6 nm and 0.195, respectively. In vitro drug release study showed that the cumulative release of DMF from the DMF-loaded sPCEG-azo polymeric micelles was no more than 20% in rat gastric fluid within 10 h, whereas in the rat colonic fluids, the cumulative release of DMF reached 60% in the initial 2 h and 100% within 10 h, indicating that the DMF-loaded sPCEG-azo polymeric micelles had excellent colon-targeted property. The DMF-loaded sPCEG-azo polymeric micelles had no significant cytotoxicity on colon cancer cells in phosphate buffered solution (PBS) and rat gastric fluid. In rat colonic fluid, the micelles showed significant cytotoxic effect on colon cancer cells. The blank sPCEG-azo polymeric micelles (without DMF) showed no cytotoxic effect on colon cancer cells in rat colonic fluids. In conclusion, the DMF-loaded sPCEG-azo polymeric micelles show colon-targeted DMF release and anti-tumor activity, providing a novel approach potential for colon cancer therapy.

Statement of Significance

Colon-targeted drug delivery and circumventing drug resistance are extremely important for colon cancer chemotherapy. Our previous work found that dimethyl fumarate (DMF), the approved drug by the FDA for the treatment of multiple sclerosis, exhibited anti-tumor activities on colon cancer cells (*Br J Pharmacol. 2015 172(15):3929-43.*). Based on the pharmacological properties of DMF and azo bond in olsalazine chemical structure, we designed azo polymeric micelles for colon-targeted dimethyl fumarate delivery for colon cancer therapy. We found that the DMF-loaded sPCEG-azo polymeric micelles showed colon-targeted DMF release and anti-tumor activities, providing a novel approach potential for colon cancer therapy.

1. Introduction

Colon cancer is one of the most prevalent diseases, and remains a significant cause of morbidity and mortality worldwide. Despite advances in chemotherapy, resistance to the anti-cancer drugs is still the greatest challenge in the management of colon cancer.

Abbreviations: DMF, dimethyl fumarate; OLZ, olsalazine; PCL, polycaprolactone; mPEG, methoxypolyethylene glycols; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; DMAP, 4-dimethylaminopyridine; sPCEG, star-PCL-mPEG; sPCEG-azo, star-PCL-azo-mPEG; TEM, transmission electron microscopy.

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The main drugs for chemotherapy of colon cancer include 5-fluorouracil (5-FU), capecitabine, tegafur, irinotecan, and oxaliplatin etc. However, resistance to these chemotherapies has arisen and the molecular mechanisms include decreased intracellular drug concentration, altered metabolism, or alterations of targets for the therapy [1]. Recent studies suggest that inducing necroptosis of colon cancer cells and colon-targeted drug delivery could increase the efficacy of anticancer drugs and decrease the drug resistance [2–4].

Necroptosis is a type of cell death different with apoptosis [5]. Necroptosis is characterized with (1) a morphology of necrotic cell death; (2) loss of plasma membrane integrity; (3) loss of mitochondrial membrane potentials; (4) elevation of reactive oxygen species; and (5) that the cell death was prevented by a small molecule, necrostatin-1 [5]. Han et al. proved that inducing necroptosis had a similar potency toward drug-sensitive cancer cell lines (MCF-7 and HEK293) and their drug-resistant lines overexpressing P-glycoprotein, Bcl-2, or Bcl-x(L), which account for most of the clinical cancer drug resistance [5]. Grassilli et al. reported that, in drug-resistant colon carcinoma cells, induction of necroptosis by GSK3B silencing resensitized drug-resistant cells to chemotherapy [6]. Therefore, induction of necroptosis would be an approach to circumvent cancer drug resistance.

Dimethyl fumarate (DMF) is the methyl ester of fumaric acid and has been approved by the FDA and European Medicines Agency as a new oral drug for the treatment of multiple sclerosis [7,8]. DMF also exhibits anti-tumor effects, for instance, it inhibits melanoma growth and metastasis [9] and induces apoptosis in HT29 colon carcinoma cells [10]. Our previous work found that DMF but not its metabolite MMF induced necroptosis in colon cancer cells through a mechanism involving the depletion of GSH, increase of ROS and activation of MAPKs [11], indicating that DMF would be a necroptosis inducer which circumvent colon cancer drug resistance. However, it should be noted that DMF could be easily hydrolyzed to mono-methylfumarate (MMF) which showed no cytotoxic effect on colon cancer cells [11]. Therefore, it must be avoided that DMF was hydrolyzed in stomach and small intestine if DMF was attempted to be developed as a local chemotherapeutic drug for colon cancer.

Presently, several strategies for colon-specific drug delivery have been explored, including the techniques dependent on pH, time, pressure and/or bacteria [12]. Colonic microfloras consist of anaerobic bacteria that are only present in the colon region and secrete specific biodegradable enzymes, this feature can be exploited to target colonic drug release. Olsalazine (OLZ) is a dimer of 5-aminosalicylic acid that are linked via an azo bond. When OLZ reaches the colon part, it is cleaved by azoreductase specifically secreted by colonic bacteria [12]. Azo bond in OLZ structure indicates that OLZ can be used as a bacteria-triggered, colon-targeted materials. Therefore, in the present study, we design DMF-loaded OLZ-linked polymer micelles (Fig. 1A) and investigate the DMF release and anti-tumor activity of these micelles. Our work will provide a novel approach for colon cancer therapy.

2. Materials and methods

2.1. Materials

ε-Caprolactone (ε-Cl), erythritol, and all the catalysts were purchased from Aladdin® company (Shanghai, China). mPEG (Methoxypolyethylene glycols) (Mn = 2 kDa) was purchased from Energy Chemical. Co. Ltd (Shanghai, China), Olsalazine Sodium (OLZ-Na) was purchased from Jusheng Technology. Co. Ltd (Hubei, China). OLZ-Na was acidized with hydrochloric acid and the product olsalazine was dried with vacuum at 60 °C. All other chemicals were purchased from commercial supplier.

2.2. Synthesis procedures

2.2.1. Synthesis of the star-shape PCL (polycaprolactone)

The star-shape PCL was synthesized by ring-opening polymerization as described in previous work with modification [13]. ε-Cl was purified by reduced pressure distillation after dried with 4A molecular sieve and added into a 100 mL round-bottom flask followed by adding $Sn(oct)_2$ as catalyst and erythritol as initiator. The flask together with its content was degassed under vacuum for 5 h at room temperature, then the ring-opening polymerization reaction was processed at 140 °C for 6 h. After the reaction system cooled down, the products were dissolved in dichloromethane (DCM) and precipitated with cold ethyl alcohol before filtration. Finally, the star-shape PCL polymers were obtained after vacuum drying for 24 h.

2.2.2. Synthesis of methoxypolyethylene-olsalazine (mPEG-OLZ)

The polymerization procedures were as following: mPEG and excess olsalazine (OLZ) were dissolved in N,N-dimethylformamide followed by adding 1-(3-dimethylaminopropyl)-3-ethylcarbodii mide hydrochloride (EDC) and 4-dimethylaminopyridine (DMAP) as catalysts into the reaction system, the reaction processed at 35 °C for 48 h. Then, the product was precipitated with cold diethyl ether before filtration and redissolved in N, N-dimethylformamide, the solution was transferred into a dialysis tube (MWCO = 1 kDa) and immersed in deionized water to remove the excess catalysts and the un-reacted OLZ. Finally, the mPEG-OLZ polymers were obtained after lyophilized.

2.2.3. Synthesis of mPEG-COOH

The mPEG-COOH was synthesized as described in previous work [14]. In brief, mPEG, butanedioic anhydride and the catalyst DMAP were added in a two-neck round-bottom flask and dissolved in dichloromethane, then the reaction processed at room temperature for 24 h. Next, the reactant solution was precipitated with cold diethyl ether before filtration. Finally, the products were obtained after vacuum drying for 24 h.

2.2.4. Synthesis of star-PCL-azo-mPEG (sPCEG-azo) and star-PCL-mPEG (sPCEG)

To synthesize the polymers with azo linkage or without azo linkage, the star-shape PCL and excess mPEG-OLZ or mPEG-COOH were dissolved in N,N-dimethylformamide followed by adding EDC and DMAP. The reaction processed at 35 °C for 48 h. Then, the reactant solution was precipitated with cold diethyl ether before filtration, and the product was redissolved in N,N-dimethylformamide. The solution was transferred into dialysis tube (MWCO = 3.5 kDa) to remove the unreacted reactants, catalysts, and N,N-dimethylformamide. Finally, the star-PCL-azo-mPEG (sPCEG-azo) polymers and star-PCL-mPEG (sPCEG) polymers were obtained after lyophilized. The total synthesis routes of the star-shape amphiphilic polymers were shown in Fig. 1B.

2.3. Assembly of polymeric micelles

The blank micelles of sPCEG-azo and sPCEG polymers were fabricated by the evaporation method. In brief, the polymer solution containing 20 mg sPCEG-azo or sPCEG in 20 mL tetrahydrofuran (THF) was added dropwisely into 40 mL deionized water with the rotor stirring. The micelle solution was filtered by 0.45 μm organic filter membrane after evaporating off THF at room temperature. The method to fabricate dimethyl fumarate (DMF)-loaded micelles was same as that for the blank micelles. The anticancer