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Stimulus-responsive supramolecular vesicles with effective anticancer activity prepared by cyclodextrin and ftorafur

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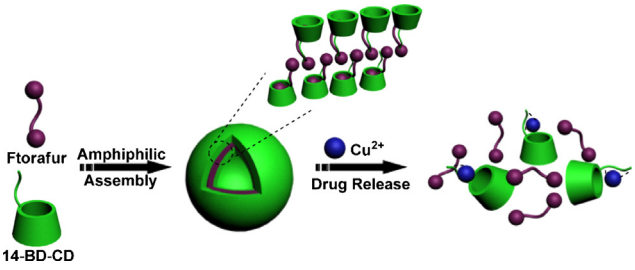
HIGHLIGHTS

- The supramolecular vesicles constructed by cyclodextrin–ftorafur supramolecular amphiphiles are reported.
- The mechanism of the vesicles formation was studied by various methods.
- The effect of copper ions on the vesicles was discussed.
- The antitumor effects of the ftorafur-loaded vesicles on human colon carcinoma cell lines HT-29 were studied in detail.

GRAPHICAL ABSTRACT

The ftorafur vesicles constructed by cyclodextrin–ftorafur supramolecular amphiphile was firstly reported here, the morphology, size, formation mechanism, stimulus responsive property and antitumor effect of the vesicles were investigated.



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ABSTRACT

Vesicles directly prepared from cyclodextrin–ftorafur supramolecular amphiphiles were reported. Ftorafur can be efficiently encapsulated in the cyclodextrin cavities embedded in the vesicle membrane. The morphologies and diameters of the vesicles were identified in detail by transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM) and dynamic light scattering (DLS). X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), UV–vis spectrum, ^{1}H NMR and 2D NMR ROESY were further employed to study the formation mechanism of the vesicles. Various morphologies were detected when different host molecules were employed as the hydrophilic moieties of the vesicles' building blocks. Copper ions could arouse the simultaneous release of ftorafur from the vesicles. The proliferation and cell cycle assay of colon carcinoma cell Line HT-29 were performed to evaluate the anticancer effects of the ftorafur-loaded vesicles and natural ftorafur, respectively. The ftorafur-loaded vesicle system exhibits a better anticancer effect than the sole ftorafur.

1. Introduction

Drug-loaded nanocarriers with proper sizes can enter the tumor interstitial space since the solid tumor tissues have high permeability vasculature, while the limited lymphatic drainage of tumor tissues allows them to stay there, this phenomenon is termed as enhanced permeability and retention (EPR) effect [1,2]. The EPR

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effect can lead to the spontaneous accumulation of drug carriers on the desired sites, where the pharmaceutic agent can be released from the carriers finally. This phenomenon results in selective targeting delivery of the active drug, which can enhance the drug effectiveness. In contrast, natural low molecular drugs can return to the blood circulation by diffusion rather than retain in tumors. The size of carriers might be a critical factor for controlling the accumulation of drug-loaded carriers in the targeting zone. The nanocarriers with the diameter less than 200 nm are believed to be more effective in passive target and with a higher intracellular uptake than free drugs [3,4].

Ftorafur, as an orally active prodrug of the anticancer drug 5-fluorouracil, is widely used in various carcinoma treatment, especially for gastrointestinal tumors [5]. Ftorafur has a good therapeutic efficacy, whereas its short biological half-life and extensive biodistribution greatly limit its applications on initial state. High doses are needed in order to improve anticancer effect, however severe side effects will occur at the same time. Drug delivery vehicles and controlled-release materials are considered as favorable means in overcoming the drug's natural shortcomings [6]. Most of these strategies are favorable but also regarded sophisticated or with tedious synthesis steps. Here, we report a vesicular system, which is directly prepared from cyclodextrin–ftorafur supramolecular amphiphiles. Ftorafur, as the hydrophobic end of the supramolecular amphiphile, can be also regarded as the carried drugs by the vesicles. The vesicles loaded with ftorafur are likely to be suitable candidate to solve the drawbacks of ftorafur through EPR effect [7].

Our team has previously reported a lot of works on the topic of cyclodextrin (CD) vesicles loading with drugs, for example: paclitaxel or UR-144 can play a good role in acting as the hydrophobic end of the supramolecular amphiphile [8,9]. Herein, the diamine modified CD, mono-(6-dexoy-6-butanediamine)-β-cyclodextrin (14-BD-CD), was synthesized (Scheme 1). 14-BD-CDs have the ability to encapsulate the therapeutic agent ftorafur in the cavities and further to form vesicles. Various morphologies were detected when different host molecules were employed as the hydrophilic moieties of the vesicles' building blocks. Diamine group can tune the vesicles morphology change through the complexation between diamine and copper ions, accompanying with ftorafur release. Moreover, the vesicles loading with ftorafur exhibit better anticancer effects than parent ftorafur through the proliferation and cell cycle assay of colon carcinoma cell Line HT-29. We believe that our method will provide new possibilities for improving drug efficacy through novel drug formulation and delivery.

2. Experimental

2.1. Materials

β-CD, OTs-β-CD, HP-β-CD and SA-β-CD, triply recrystallized in distilled water and then dried under vacuum for 12 h at 50 °C before using, were purchased from Binzhou Zhiyuan Biotechnology Co. Ltd., China. 12-PD-CD, 13-PD-CD and 14-BD-CD were synthesized according to the literature [10]. The substitution degrees are all 1. Ftorafur was obtained from Jinan Yingsheng Co. Ltd., China. All other organic reagents were of analytical quality without further purification and were all commercially available from Sinopharm Chemical Reagent Co. Ltd., China. Human colon carcinoma cell lines HT-29 was obtained from Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, P.R. China), it was maintained in RPMI-1640 medium (Gibco BRL, Gaithersburg, MD), containing 10% fetal bovine serum (FBS) and antibiotics (100 mg/mL penicillin and 100 mg/mL streptomycin) with 5% CO_2 at 37 °C.

2.2. Synthesis of β-CD derivatives

β-CD derivatives were synthesized according to the literature. The synthesis process is shown in Scheme 1. Briefly, mono-(6-*O*-paratoluensulfonyl)-β-CD (2.578 g, 2 mmol) and 1,4-tetramethylenediamine (0.22 g, 2.5 mmol) were dissolved in 30 mL *N*-methylpyrrolidone, and then 1 mL triethylamine was dropped slowly in the solution as catalyst. The resulting mixture was stirred for 10 h in oil bath at 70 °C and monitored by TLC (isopropanol:water:ammonia water = 5:2:1). Then the reaction solution was cooled spontaneously. White precipitate appeared after the cooled solution was poured into 50 mL acetone. The precipitate was filtered by vacuum filtration and was washed three times with acetone. The product **14-BD-CD** was obtained by purifying the rude product through silica gel column chromatography with a mixed eluent (isopropanol:water:ammonia water = 10:2:1), the yield was 91%, white powder, R_f = 0.51. ^{1}H NMR (400 MHz, D_2O, 300 K, DHO, δ ppm): 4.96 (d, J = 4.0 Hz, 1*H*, H-1), 4.70 (s, D_2O), 3.85 (t, J = 20.0 Hz, 1*H*, H-3), 3.77 (d, J = 4.0 Hz, 2*H*, H-6), 3.75–3.72 (m, 1*H*, H-5), 3.55–3.52 (m, 1*H*, H-2), 3.49 (t, J = 20.0 Hz, 1*H*, H-4), 2.85 (t, J = 12.0 Hz, CH_2, 2*H*), 2.55 (t, J = 16.0 Hz, CH_2, 2*H*), 1.58–1.52 (m, CH_2, 2*H*), 1.51–1.43 (m, CH_2, 2*H*). FT-IR (KBr pellet, υ cm^{-1}): 3451.94 (vs, sh, ν_{NH_2}), 2933.53 (vs, sh, ν_{CH_2}), 1635.58 (w, m, δ_{NH_2}), 1161.97 (m, sh, ν_{C-N}), 1038.90 (s, sh, δ_{CH}), 595.29 (w, δ_{CH_2}). ESI-MS Calcd. for $C_{46}H_{79}N_2O_{34}$ m/z 1204.11, found m/z 1204.46.

2.3. Analytical instruments and methods

TEM images were carried out on a JEM-100CX electron microscope from JEOL Ltd. SEM pictures were gotten with a Hitachi S-4800 scanning electron microscope. The samples for TEM detection were dropped in the copper wire mesh and stained by the phosphotungstic acid. Then the samples were dried under the ultraviolet lamp. The samples of SEM measurement were obtained directly by sticking the TEM samples to the basement and then sprayed by the gold. AFM testing was conducted with a Veeco Nanoscope Multimode III SPM and operated in tapping contact mode at ambient temperature. The AFM sample was dropped on the smooth silicon wafer and dried by freeze drying for 5 days. The average diameter of vesicles was recorded by DLS measurement with a Wyatt QELS Technology DAWN HELEOS instrument, which used a 12-angle replaced detector in scintillation vial and a 50 mW solid-state laser. The water for preparation samples of DLS was filtered by 0.45 μm filter and samples of DLS were also filtered by 0.45 μm filter before testing. The X-Ray powder Diffraction experiment was performed on a German Bruker/D8 ADVANCE diffractometer with Cu Kα radiation. The supramolecular inclusion of 14-BD-CD and ftorafur was prepared in the same way of FT-IR sample preparation. The physical mixture of host and guest was obtained by mixing the powder of 14-BD-CD and ftorafur quickly before testing in order to avoid the formation of supramolecular inclusion. IR spectrum was obtained on an Avatar 370 FT-IR Spectrometer with KBr pellet method at room temperature. The solid supramolecular inclusion of 14-BD-CD and ftorafur was gotten by rapid freeze drying of the inclusion solution for staying the inclusion in suit state. For the preparation of 14-BD-CD and ftorafur mixture, the equal mole 14-BD-CD and ftorafur were grinded with KBr separately, then the mixture was obtained by mixing the grinded host and guest molecule quickly in case of forming the supramolecular inclusion. ESI-MS spectrum was performed on API 4000 MS equipment with MeOH as the solvent. ^{1}H NMR spectra was obtained on an API Bruker Avance 400 M NMR with D_2O as the solution at room temperature. 2D NMR ROESY experiments were recorded using an API Bruker Avance 300 M NMR at ambient temperature referenced to the solvent peak at δ = 4.70 ppm in D_2O. UV–vis curves were obtained at room temperature with a