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Research paper

The preparation and characterization of lactone form of 10-hydroxycamptothecin-layered double hydroxide nanohybrids

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ABSTRACT

10-Hydroxycamptothecin (HCPT) as a hydrophobic anticancer drug brings many challenges in the clinical applications due to its poor water solubility and its facile structure transformation to inactive structure. In this work, the lactone form of HCPT–LDH nanohybrids with high drug loading was prepared by a two-step method. Firstly, the carboxylate form of HCPT-intercalated LDH was prepared by a coprecipitation method using a microchannel reactor. Secondly, dilute acetic acid was added into the as-precipitates to perform a structure recovery of HCPT in the nanohybrids from carboxylate form to bioactive lactone form because the lactone–carboxylate equilibrium of HCPT is reversible and pH-dependent. The dispersity of HCPT–LDH nanohybrids was obviously improved as compared with particles prepared by conventional method, and the average particle size increased with the drug loading. It was found that the content of HCPT in the nanohybrids with bioactive lactone form reached 95% after treated with acetic acid. For cancer cells, the lactone form of HCPT–LDH was found to be significantly more potent than raw HCPT, and it provided an important foundation for the development and application of nanohybrid delivery system.

1. Introduction

The pentacyclic alkaloid, 10-hydroxylcamptothecin (HCPT), isolated from the Chinese tree, *Camptotheca acuminata*, inhibits the activity of topoisomerase I and has a broad spectrum of anticancer activity in vitro and in vivo (Ping et al., 2006). However, as shown in Scheme 1, HCPT has a poor water solubility and more importantly, it facilely transits from the bioactive lactone form (I) into the inactive carboxylate structure form (II) via hydrolysis even at physiological conditions (Kunadharaju and Savva, 2008). Therefore, the efficient delivery of HCPT is an extreme challenge up to now. Nanosized vehicles including liposomes (Chang et al., 2009; Volodkin et al., 2009), water soluble polymers (Liu et al., 2009), dendrimers (Guillaudeu et al., 2008), vesicles (Soussan et al., 2009), polymernanoparticles (Tong and Cheng, 2008), and some inorganic materials (Lu et al., 2009; Pang et al., 2013) are investigated as the carriers of the hydrophobic drugs to greatly enhance their water solubility and stability, prolong their circulation in blood compartments, and target cancerous tissues by passive accumulation via tumors' enhanced permeability and retention (EPR) effect (Shen et al., 2010).

Layered double hydroxides (LDH), with structurally positively charged layers and interlayer balancing anions, were applied widely in different fields (Tong et al., 2010; Basu et al., 2014). In recent years they were frequently investigated as drug delivery nanocarriers because their interlayer gallery could be employed as drug vessels with controlled releasing properties (Ambrogi et al., 2001; Rives et al., 2014). The water soluble anionic drugs can be intercalated into the hydrophilic gallery of pristine LDH via ion exchange (Meyn et al., 1990), reconstruction (Dong et al., 2010) and co-precipitation (Liu et al., 2008), while the charge-neutral or poorly water-soluble drugs, such as HCPT can be intercalated into the hydrophobic gallery of modified LDH usually with surfactants (You et al., 2001; Bruna et al., 2006). However, the LDH, its sole role is to make the vehicles, is the major component while the bioactive drug is the minor component in nanomedicines. In nanoparticles, the drug contents are generally not greater than 10% (Tyner et al., 2004). The HCPT–LDH nanohybrids with high drug loading can be prepared by a coprecipitation method (Liu et al., 2008), but the loaded HCPT in nanohybrids showed a poor bioactivity.

Herein, HCPT–LDH nanohybrid was firstly prepared with co-precipitation method as reported (Liu et al., 2008), and then the sediment was treated with diluted acetic acid to enhance the bioactivity of the loaded drug. Because the lactone–carboxylate equilibrium (Scheme 1) is reversible and pH-dependent, the treatment in the presence of acetic acid promotes a conversion from the inactive carboxylate structure (II) into bioactive lactone form (I) (Kunadharaju and Savva, 2008).

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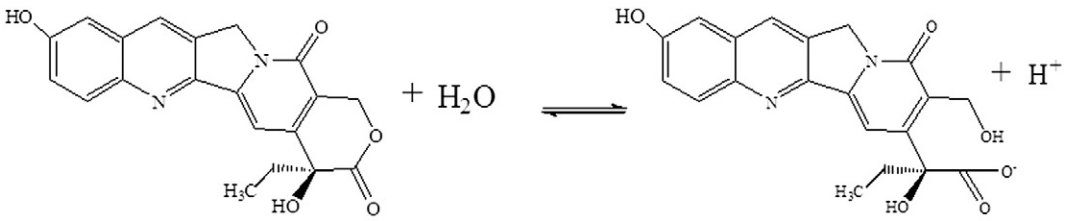

(I) lactone form **(II) carboxylate form**

Scheme 1. The conversion of the lactone form of HCPT into its carboxylate form.

2. Experimental

2.1. Synthesis of $HCPT_C$–LDH and $HCPT_L$–LDH

$HCPT_C$ (carboxylate form of HCPT)-LDH nanohybrids were synthesized by coprecipitation method. A mixed salt solution containing 5.94 g (0.020 mol) of $Zn(NO_3)_2 \cdot 6H_2O$ and 3.75 g (0.010 mol) of $Al(NO_3)_3 \cdot 9H_2O$ in 60 ml of deionized H_2O was prepared. A series of HCPT with different weight (0.0089–0.890 g) (Hubei Haobo Co., Ltd., China) were respectively dissolved by dipping 0.1 mol/l aqueous solution of NaOH and then water was added to reach a solution volume of 60 ml. Then the mixed salt solution and alkaline solution containing HCPT were mixed by a microchannel reactor and a suspension was obtained. The precipitate was aged for 3 h in the mother solution at 40 °C and then centrifuged and washed with deionized water for two times. The centrifuged product held in a glass bottle was peptized at a constant temperature of 60 °C in an oven for about 24 h to obtain $HCPT_C$–LDH nanohybrid. $HCPT_C$–LDH nanohybrid was dispersed in deionized water and then 5% (by volume) diluted acetic acid was added and the pH value of suspension was adjusted to 5.5. After stirring at 60 °C for 12 h, the precipitate was centrifuged and then dried, and the so-obtained sample was denoted as $HCPT_L$ (lactone form of HCPT)–LDH. The drug loading of the sample changed little (<11%) before and after the treatment by dilute acetic acid.

2.2. Determination of HCPT loading

The amount of HCPT loaded into the hybrids, A_{in}, was determined by UV–vis spectroscopy using the following method. 10 mg of the hybrid sample was dissolved in 10 ml HCl solution (1 mol/l), which was followed by addition of 40 ml ethanol. The concentration of HCPT in solution was determined by monitoring the absorbance at $\lambda_{max} = 380$ nm with a UV–vis absorption spectrophotometer, and the concentration was calculated by regression analysis according to the standard curve obtained from a series of standard solution of HCPT. The A_{in} value was obtained according to the weight ratio of the HCPT in the solution and the nanohybrid used.

2.3. Determination of the release rate of HCPT from the nanohybrids

The HCPT release examinations were performed at 37 °C in 0.1 M phosphate buffer solution (PBS) with a pH value of 7.2 or ethanol–water mixture (3:7 by volume). 50 mg of the HCPT–LDH nanohybrid was placed into 500 ml buffer solution or ethanol–water mixture under stirring at 37 °C. Aliquots (4 ml) of the suspension were withdrawn at predetermined time intervals and filtered through a 0.45 μm syringe filter. The absorbance was measured at $\lambda_{max} = 380$ nm by a UV–vis spectrophotometer to obtain the HCPT concentration, and then to calculate the release percentage (X_t) of HCPT. The release percentages of HCPT from the composite were plotted versus time (t) to examine the release rate of HCPT from the nanocomposite.

2.4. HPLC analysis of HCPT

The lactone and carboxylate forms of HCPT were separated and quantified with the Dionex Summit high performance liquid chromatography (HPLC) System equipped with P680A HPG-2 High-Pressure Gradient Pump and UVD 170U HPLC UV–vis Detector. Samples were dispersed in dimethyl sulfoxide (DMSO) and stirred for 12 h, and then the dispersion was filtered through a 0.22 μm syringe filter. The concentration of HCPT is about 0.15 mg/ml. The mobile eluent phase was 70% phosphate buffer (pH value = 6.5, 25 mM), 23% methanol, and 7% tetrahydrofuran with the detection wavelength of 382 nm. The lactone fraction was calculated by the equation of $f_{lactone} = A_{lactone}/A_{total}$, in which $A_{lactone}$ refers to the area of the lactone peak and A_{total} is the total peak area of the lactone and carboxylate forms.

2.5. In vitro cytotoxicity against HCT-116 and Colo-205 cells

For the cytotoxicity analysis of free HCPT, pristine LDH and HCPT–LDH against HCT-116 and Colo-205 cells, cells were seeded in a 96-well plate at a density of 104 cells per well and cultured in 5% CO_2 at 37 °C for 72 h. Then, free HCPT dispersed in DMSO and PBS, respectively, pristine LDH and HCPT–LDH dispersed in PBS, and then the cells were incubated in 5% CO_2 at 37 °C for 72 h. The concentrations of free HCPT and encapsulated HCPT in nanohybrids were 0.001, 0.004, 0.012, 0.037, 0.111, 0.333, 1.0 and 3.0 μg/ml, respectively. The concentrations of pristine LDH were 0.5, 1.4, 4.1, 12.3, 37, 111, 333 and 1000 μg/ml, respectively. Cell viability was determined by CCK8 assay. Each data point is represented as mean value ± standard deviation (SD) of eight independent experiments ($n = 8$, n indicates the number of wells in a plate for each experimental condition). IC50 values were used to value the cytotoxic activity of different samples.

2.6. Characterizations and Instrumentations

Powder X-ray diffraction (XRD) patterns were obtained on a D/max-rA model diffractometer with Cu$K\alpha$ radiation (40 kV and 80 mA). Transmission electron microscopy (TEM) images were obtained on a JEM-2100 transmission electron microscope. Fourier transform infrared (FT-IR) spectra were recorded in KBr dispersion on a Bruker Vector 22 spectrometer in air at room temperature.

3. Results and discussion

3.1. Preparation of $HCPT_C$–LDH and $HCPT_L$–LDH nanohybrids

In the past years, nonionic and poorly water-soluble drug was usually first encapsulated in an anionic micelle and then the negative charge on the surfactant allows the uptake of the drug-loaded micelle between the sheets of the LDH by an ion exchange process (Tyner et al., 2004; Han et al., 2005). By these methods, drugs could be intercalated into the gallery of LDH successfully, but drug loading is quite low. In this