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The synthesis and application of heparin-based smart drug carrier

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ABSTRACT

Heparin based polymer drug which could self-assemble into sphere micelle in water was firstly prepared by grafting paclitaxel (PTX) into the hydroxyl of heparin via aconitic bond as pH sensitive spacer. Positive charged drug DOX·HCl and cationic folic acid (CFA) can be further loaded into the polymer drug via electrostatic interaction in aqueous solution so as to prepare smart drug carrier. The drug carrier was able to release more PTX and DOX at pH 4.8 than that at pH 7.4, exhibiting pH sensitivity for two drugs. Furthermore, tumor cell cytotoxicity test proved it possessed significant cytotoxicity against tumor cells MDA-MB-231 as well as its active tumor targeting ability resulting from the loading of CFA. Cellular uptake and intracellular distribution were further revealed by confocal laser scanning microscopy (CLSM). In conclusion, this paper not only provided a simple strategy but also indicated heparin is a versatile platform for the design of smart drug carrier. The as-prepared drug carrier also showed promising potential in chemotherapy.

1. Introduction

Polymer micelles formed by amphiphilic polymers have shown promising potential to be drug carriers for chemotherapy in anti-tumor treatment after several decades' development (Bae, Fukushima, Harada, & Kataoka, 2003; Davis, Chen, & Shin, 2008; Yokoyama et al., 1992; Kabanov, Vinogradov, Suzdaltseva, & Alakhov, 1995; Hu et al., 2010), which offer many significant advantages for anti-tumor drugs over traditional small molecule therapeutics such as improved aqueous solubility by encapsulating the drugs into the hydrophobic core(Van et al., 2010) and sustainable release behavior (Larson & Ghandehari, 2012) so that it can preliminarily meet fundamental clinical requirement of chemotherapy, thereby increasing anti-tumor efficacy and avoiding undesired side effects, toxicity or organ damage (Wang, Langer, & Farokhzad, 2012a; Timko et al., 2011).

Recently, researchers introduced various functions such as passive and positive tumor targeting ability, controlled drug release and so on (Miyata, Christie, & Kataoka, 2011; Yoo & Park, 2004; Yasukawa et al., 1999; Pasut & Veronese, 2009; Chipman, Oldham, Pezzoni, & Singer, 2006; Hu, Tang, & Chu, 2014a; Shantanu, Aswathy, Athulya, Sakthi, & Veena, 2014) into polymer micelles in order to endow some smart drug release behaviors in chemotherapy treatment. Each function possesses its unique advantage on further increasing drug efficacy and avoiding side effects (Hu, Fang, Luk, & Zhang, 2014b; Petros & DeSimone, 2010). Furthermore, it is intended to assemble functions as many as possible into one drug carrier to form a drug carrier so that it can play the best role in reducing toxicity and improving efficacy. Recently, Abeylath, Ganta, Iyer, and Amiji (2011) designed a polymeric nanosystem which gains targeting and imaging ability at the same time via physical conjugating. However, there are still some difficulties including the lack of modification sites, the tedious synthetic protocol and so on, to prepare smart drug carrier from traditional amphiphilic copolymers. Therefore, it remains an important task for researchers to design and prepare a novel amphiphilic copolymer which can not only possess abundant modification sites but also be endowed with various functions via simple modification.

On the other hand, polymer drugs which directly connect hydrophobic drugs with hydrophilic polymer have also attracted an increasing attention in recent years (Hu et al., 2012; Filippov et al., 2013). It is believed that polymer drugs can significantly increase drug loading content (DLC)via chemical combination compared with traditional polymer carrier via physical encapsulation (Yu et al., 2013), and the biocompatibility may also improve due to the spare of hydrophobic polymer chain. However, the pharmacokinetics and pharmacodynamics of polymer drug are unclear. In order to avoid this risk, it is necessary to use biodegradable bond to link drug and polymer so that the drug can be released from the polymer chain on specific sites. Zhong et al. (2013) introduced

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an acetal-linked PTX polymer drug which can release the drugs on tumor sites due to the hydrolysis of acetal. Moreover, it is also desirable to endow multiple functions to polymer drug to increase drug efficacy and avoid side effects.

In our previous work (Ye et al., 2014), a novel binary-drug loaded micelle based on PCL-heparin conjugate was synthesized via EDCI/NHS. This micelle was able to load two kinds of drugs, paclitaxel (PTX) and doxorubicin hydrochloride (DOX·HCl), in its hydrophobic core and hydrophilic shell respectively. Moreover, it showed pH sensitive release behavior of DOX. Subsequently, cationic folic acid (CFA) was also introduced into its negative shell via electrostatic interaction in order to achieve positive tumor targeting ability (Fang et al., 2013; Hu et al., 2014c). These versatile functions-endowing processes as well as easy preparation indicated that heparin was a good platform upon which the drug carrier could be constructed via simple modification (Gu, Faig, Abdelhamid, & Kathryn, 2014). However, some functions such as pH sensitivity of PTX was still absent and the drug loading content was relatively low in this micelle. Besides, the PCL blocks in the conjugate might also have the risk of reducing drug efficacy and even producing drug resistance due to its sustained degradation kinetics inside the body over a period of days to weeks (Shuai, Ai, Nasongkla, Kim, & Gao, 2004; Wang et al., 2012b). In this point of view, a better heparin based platform with more biocompatibility and multi-pH sensitivity as well as high DLC should be processed (Nguyen & Alsberg, 2014).

Herein, we directly linked heparin and paclitaxel by *cis*-aconitic bond to synthesize HEP–PTX polymer drug. This polymer drug can also self-assemble into nanometer size micelle in water with a hydrophobic PTX core and a negative charged hydrophilic shell. According to similar strategy, this polymer drug was able to absorb DOX·HCl and the tumor target molecule CFA via electrostatic interaction. Simultaneously, pH sensitivity of PTX was achieved since the endowed *cis*-aconitic linker is pH sensitive and would be broken to trigger PTX releasing in acidic tumor environment. Thus, a smart drug carrier which assembles pH sensitivity of both PTX and DOX, positive and passive tumor targeting ability into one carrier was achieved via simple manipulation. On the other hand, this formed drug carrier based on HEP–PTX polymer drug provided relatively higher DLC and biocompatibility than previous PCL-heparin drug carrier. In summary, heparin which can not only be chemically grafted with drug molecules but also be loaded with cationic substances via electrostatic interaction served as a good platform in this study to construct both polymer drug and drug carrier, while this work also offered a versatile strategy for the preparation of drug carrier via simple manipulation. Besides, the as-prepared drug carrier is expected to be able to play the best role in increasing drug efficacy and avoiding side effect in chemotherapy resulting from the various functions assembled in the carrier.

2. Experiment

2.1. The preparation of HEP–PTX based drug carrier

2.1.1. Materials and measurements

PTX and folic acid were both bought from Shanghai Jinhe Biotech Co., Ltd, and DOX·HCl was purchased from Beijing Huafeng United Technology Co., Ltd. Heparin(HEP, MW 5000-12000) was bought from Beijing Shijizhongwei Technology Co., Ltd. 1-Ethyl-3-(3-dimethylaminopro-pyl) carbodiimide (EDC), *N*-hydroxysuccin-imide (NHS), diisopropylethylamine(DIPEA) and cis-aconitic anhydride were all bought from Sigma-Aldrich, USA and used as received. All of other chemical reagents and solvents used were of analytical grade.

FTIR measurement was conducted on a Shimadzu IR Prestige21 spectrometer and ^{1}H NMR was carried on Bruker ARX 400 apparatus with TMS as internal standard. UV spectra were measured on a HITACHI U-280 UV–vis spectrometer.

2.1.2. The preparation of *paclitaxel*-cis-*aconitic compound*

Firstly, *cis*-aconitic andydride (0.36 g, 0.002 mol) and DIPEA(0.8 mL)were dissolved in DMF(5 mL) to activate the andydride bond in a flask. PTX (2.00 g, 0.002 mol) was dissolved in DMF (10 mL) in vial and then dropped into the solution. The mixture was stirred at room temperature for 5 h. After reaction, the product was precipitated by water, filtrated and dried by lyophilization (0.259 g, yield = 11.0%).

2.1.3. The preparation of HEP–PTX polymer drug

PTX-*cis*-aconitic compound(0.15 g, 7.3×10^{-5}mol), heparin(0.14 g, 1.4×10^{-5} mol), EDC(0.14 g, 7.3×10^{-5} mol), DMAP(0.09 g, 7.3×10^{-5} mol) and triethylamine (0.09 g, 8.9×10^{-4} mol) were all dissolved in DMF(20 mL) and reacted for 8 h at 50 °C. The resulting solution was firstly dried by rotary evaporator and then refined by dialyzing to water. After centrifugation, the supernatant liquor was further dried by lyophilization for 12 h to get HEP–PTX polymer drug (26.9 mg, yield = 9.3%).

2.1.4. The synthesis of drug carrier

The synthesis of CFA can be found in our previous work (Hu et al., 2014c). The drug carrier was obtained after loading of CFA and DOX·HCl into HEP–PTX polymer drug. 2 mL aqueous solution (1 mg/mL) of DOX·HCl with prepared CFA (0.1 mg) was dropped into 20 mL aqueous solution (1 mg/mL) of HEP–PTX polymer drug slowly. The mixed solution was stood without stirring at room temperature for 4 h. Thereafter the solution was transferred into dialysis tubing (MWCO 3500) and dialyzed against distilled water for 12 h. The drug carrier was finally obtained by lyophilization.

2.2. The characterization of drug carrier

2.2.1. Measurement

Zeta potential was measured on a NANO ZS ZEN3600, Malvern Instruments, and the micelle morphology was observed on a JEM-3010 Transmission Electron Microscope (TEM). The dynamic laser scattering (DLS) measurement was carried on a D-63225 Langen/Hessen Laser Goniometer System, Germany. The HPLC measurement was carried on SHIMADZU LC-30A, using Diamonsil TM C18 column (250 × 4.6 mm, 5 μm). HPLC grade methanol and acetonitrile were bought from Shanghai Anpu Chemical Company.

2.2.2. Critical micelle concentration (CMC)

The CMC value was determined by fluorescence spectroscopy using pyrene as fluorescence probe at room temperature. An aliquot of 1 mL acetone solution of pyrene were transferred into 20 mL vials and the acetone evaporated to dryness. Subsequently, a series of drug carrier solutions ranging from 1×10^{-3} to 2.0 mg/mL were added to the vials to give a final pyrene concentration 6×10^{-7} mol/L. The ratios of the excitation spectra's fluorescent intensities at 373 nm and 384 nm (I_{373}/I_{384}) were calculated and plotted against the logarithm of mass concentration.

2.2.3. TEM&DLS

The morphologies of HEP–PTX polymer drug and its derided drug carrier were observed by TEM at an accelerating voltage of 200 kV. Samples were obtained by dissolved in water. The solution was stirred at room temperature overnight. After that, a drop of prepared solution(5 mg/mL) was deposited on a carbon-coated copper grid. Finally, after a thorough air-drying, the samples were photographed. Besides, samples were dissolved in water (2 mg/mL) and then filtrated before the DLS test.