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Hydroxyethyl starch conjugates for improving the stability, pharmacokinetic behavior and antitumor activity of 10-hydroxy camptothecin

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ABSTRACT

10-Hydroxy camptothecin (10-HCPT)–hydroxyethyl starch (HES) conjugates were prepared to improve the water solubility, prolong the half-life in plasma and increase the antitumor efficacy of 10-HCPT, and the structures of the conjugates were confirmed by NMR and infrared spectroscopy. The 10-HCPT conjugates showed good sustained release effect in phosphate-buffered saline (PBS), rat plasma and liver homogenate. Meanwhile, 10-HCPT–HES conjugates achieved much lower IC_{50} and higher cytotoxicity effects than the free 10-HCPT on Hep-3B and SMMC-7721 cell lines. The pharmacokinetics results of 10-HCPT–HES conjugates demonstrated that the biological half-life of 10-HCPT was increased from 10 min to 2.94 h and 3.76 h, respectively, in comparison with the commercial 10-HCPT injection. The pharmacodynamics results indicated that 10-HCPT-HES conjugate had a better antitumor efficiency against nude mouse with Hep-3B tumor than the commercial 10-HCPT injection, and the inhibition ratio of tumor was 78.3% and 31.5%, respectively, at the dose of 1.0 mg/kg. These findings suggest that 10-HCPT-HES conjugate is a promising drug delivery system providing improved long circulating effect, greater stability and better antitumor effect.

1. Introduction

10-Hydroxy camptothecin (10-HCPT) is one of the camptothecin analogues with a powerful cytotoxic effect and strong antitumor activity against gastric carcinoma, hepatoma, leukemia and head and neck tumors in clinical practice. (Grillet et al., 2011; Lorence and Nessler, 2004; Subramanian et al., 2011). It has a unique mechanism that allows it to inhibit the DNA topoisomerase I of the tumors and, at the same time, controls the proliferation of the cancer cells and induces cell death (Lee et al., 2010). However, the therapeutic application of 10-HCPT is restricted by its low aqueous solubility and poor stability *in vitro* and *in vivo* (Zhang et al., 2007). It is known that 10-HCPT has a pH-dependent equilibrium between its lactone form and carboxylate form under physiological conditions. Generally speaking, the lactone form of 10-HCPT is essential for antitumor activity, and the carboxylate form is inactive. Additionally, the carboxylate form can induce severe cumulative hematological toxicity, diarrhea and chemical or hemorrhagic cystitis, which are often formidable and unpredictable. Meanwhile, serum albumin preferentially binds the carboxylate form of 10-HCPT and accelerates *in vivo* elimination of 10-HCPT (Botella et al., 2011). Thus, how to increase the solubility, maintain the stability of the active lactone, prolong the half-life and reduce the side effects are critical for the efficacy of 10-HCPT.

Among a variety of approaches, the use of biocompatible polymer conjugated anticancer drugs is a well known and widely exploited technique to modify the properties of active agents and to improve the therapeutic response (Shakya et al., 2010; Vicent and Duncan, 2006). The idea of covalently attaching insoluble chemotherapeutic agents to a water-soluble polymer was first proposed by Ringsdorf in the mid-1970s, and polymer–drug conjugates generally exhibit a prolonged half-life, higher stability and greater water solubility (Li and Wallace, 2008). In addition, the development of multi-drug resistance (MDR) by cancer cells, which results from the expression of a p-glycoprotein pump in the cell membrane, hampers the drug action by pumping out drug

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molecules from the cytosol into the extracellular area, whereas the polymer–drug conjugates have shown an excellent ability to avoid MDR by changing the way by which they enter the cell (Feng and Chien, 2003). Many water-soluble polymers have been used as carriers to conjugate anticancer drugs, such as dextran, *N*-(2-hydroxypropyl)methacrylamide copolymer (HPMA), polyethylene glycol (PEG) or polyglutamic acid (Huan et al., 2009; Manju and Sreenivasan, 2011; Nakase et al., 2008; Pasut and Veronese, 2009; Shahnaz et al., 2010; Xie et al., 2007).

Several camptothecin conjugates have been reported by some researchers in recent years, including PEG–camptothecin conjugate (Hong et al., 2010; Paranjpe et al., 2004), polyglutamate–camptothecin conjugate (Pasut and Veronese, 2007), PEG–SN38 conjugate (Zhao et al., 2008) and PAMAM–camptothecin conjugate, and some of them have undergone clinical investigations. All of these conjugates exhibited higher water solubility, better lactone stability and antitumor effects compared with the free camptothecin. Other than improving the solubility and stability of 10-HCPT, polymer–drug conjugate can also prolong circulation time through avoiding rapid clearance by the renal and reticuloendothelial systems (RES), decrease side effects, passive target tumor tissues via the enhanced permeability and retention (EPR) effect, and improve bioavailability.

Based on our research purpose and the structure of 10-HCPT, water-soluble polymer hydroxyethyl starch (HES) was selected to form a covalent link with 10-HCPT. It is well known that HES is a semi-synthetic biodegradable polymer widely used as a plasma volume expander (Feng et al., 2007; Nanaki et al., 2012; Noga et al., 2012, 2013; Wohl-Bruhn et al., 2012) and it is degraded *in vivo* by serum α-amylase, and it offers the advantage of controllable biodegradation behavior. In addition, it has a high water solubility, low hypersensitivity and protein repellent characteristics. These excellent properties make HES an important biomedical material, and it has been tested as a substitute for PEG to extend the circulation time of drugs with a short half-life, and as a cryoprotectant for peptides, proteins and other nanoparticulate systems.

In this report, 10-HCPT–HES conjugate was conceived as novel drug delivery carrier to transport and release 10-HCPT. HES with two different average molecular weights and degree of substitution, 130 kDa/0.4 and 200 kDa/0.5, were selected. Using glycine as a spacer, 10-HCPT was chemically conjugated to HES by ester bonds and amide bonds. The *in vitro* release in PBS solution, stability in plasma and liver homogenate and cytotoxicity in Hep-3B and SMMC-7721 cancer cells were investigated. The *in vivo* pharmacokinetics in rats and pharmacodynamics in nude mice with Hep-3B cancer cells were also evaluated. The results obtained showed that 10-HCPT–HES conjugates significantly increased the solubility and lactone stability of 10-HCPT. Meanwhile, longer circulating effect in plasma, better cytotoxicity and higher antitumor effect were exhibited in comparison with free 10-HCPT.

2. Materials and methods

2.1. Material and reagents

10-Hydroxy camptothecin and a commercial 10-hydroxy camptothecin injection (1 mg/mL) were purchased from Wuhan Lishizhen Pharmaceutical Co., Ltd. (Wuhan, China). HES (130 kDa/0.4) and HES (200 kDa/0.5) were purchased from Chongqing Daxin Pharmaceutical Co., Ltd. (Chongqing, China). 1-Ethyl(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDC.HCl), 4-dimethylaminopyridine (DMAP) and *N*-hydroxysuccinimide (NHS) were purchased from Shanghai Medpep Co., Ltd. (Shanghai China). *N*-(*tert*-Butoxycarbonyl)glycine (98%), di-*tert*-butyl dicarbonate (99%), pyridine (99%), and succinic anhydride (99%) were obtained from Shanghai Darui Fine Chemical Co., Ltd. (Shanghai, China). 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was furnished from Sigma. Dichloromethane (DCM) and dimethyl sulfoxide (DMSO) were dried with 4 Å molecular sieve before use.

2.2. Animals and cell lines

Eighteen male Wistar rats weighing 200 ± 20 g were obtained from the Laboratory Animal Center of Shenyang Pharmaceutical University. Rats were housed in groups of two or three with a 12 h light/12 h dark cycle in an air-conditioned room (25 ± 2 °C), at a relative humidity of 45 ~ 60%, for 1 week. The rats were fasted for 12 h but had free access to water prior to the administration.

Twenty-four nude mice weighing 20 ~ 25 g were obtained from the Laboratory Animal Center of Shenyang Pharmaceutical University. The mice was housed in an air-conditioned room (25 ± 2 °C), at a relative humidity of 45 ~ 60%. All animal-use procedures were in accordance with the regulations for animal experimentation issued by the State Committee of Science and Technology of the People's Republic of China.

The human liver cancer cell line Hep-3B and the gastric cancer cell line SMMC-7721 were obtained from the American Type Culture Collection (Manassas, VA). They were maintained as monolayer cultures in Dulbecco's modified Eagle's medium (DMEM, Gibco, Invitrogen) supplemented with 10% fetal bovine serum (FBS) and maintained at 37 °C in a humidified incubator with 5% CO_2. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was purchased from Sigma, U.S.A.

2.3. Synthesis and characteristics of 10-HCPT–HES conjugates (He et al., 2004; Paranjpe et al., 2004; Zhao et al., 2008)

2.3.1. Synthesis of 10-HCPT–HES conjugates

The synthetic procedures for 10-HCPT–HES conjugates were described in the following steps as shown in Fig. 1: firstly, to introduce the carboxyl groups into HES, HES—COOH was synthesized by the interaction between HES and succinic anhydride; secondly, amino groups were introduced into 10-HCPT by reacting with di-*tert*-butyl dicarbonate and *N*-(*tert*-butoxycarbonyl)glycine to produce 10-HCPT–Gly (compound 4); finally, the 10-HCPT–HES conjugates were formed. Briefly, EDC (1.2466 g, 6.53 mmoL) and NHS (0.6078 g, 5.29 mmoL) were added to the solutions of HES130—COOH and HES200—COOH (5.0309 g and 4.9338 g, in anhydrous DMSO, 100 mL), respectively, to activate the carboxyl groups of HES—COOH, and the solutions were stirred continuously for 24 h at 40 °C. Subsequently, 10-HCPT–Gly (0.9980 g, 1.87 mmoL) was added to the above solutions separately, and allowed to react for 96 h at 40 °C. After the reaction was completed, the solution was added to a mixture of anhydrous ethanol and anhydrous diethyl ether (v:v = 1:1), and the precipitate was collected. To further purify the products, the precipitates were processed twice using the same precipitation process. Finally, the solids were dried under vacuum at 40 °C to give the 10-HCPT–HES (130 kDa/0.4) conjugate and the 10-HCPT–HES (200 kDa/0.5) conjugate (4.85 g, 96% yield and 4.75 g, 93% yield, respectively).

2.3.2. Characterization of 10-HCPT–HES conjugates

Following the synthesis of the 10-HCPT–HES conjugates, TOF-MS, Infrared Spectroscopy and NMR techniques were performed to confirm their structures. In addition, there are three different forms of the 10-HCPT in pH 7.4 PBS solution, such as lactone ring form, carboxylate form and the conjugate form. These three forms of the 10-HCPT were monitored simultaneously by HPLC because of their polarity differences.