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Encapsulation of honokiol into self-assembled pectin nanoparticles for drug delivery to HepG2 cells

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ABSTRACT

Self-assembled pectin nanoparticles was prepared and evaluated for delivering the hydrophobic drug, honokiol (HK), to HepG2 cells. These hydrophobic drug-loaded nanoparticles were developed without using any surfactant and organic solvent. Hydroxypropyl-β-cyclodextrin (HCD) was used to fabricate an inclusion complex with HK (HKHCD) to increase the solubility of the drug and thus facilitate its encapsulation and dispersion in the pectin nanoparticles. Investigation of the in vitro release indicated that the drug-loaded nanoparticles exhibited a higher drug release rate than free honokiol and an effective sustained-release. Cytotoxicity, cell apoptosis and cellular uptake studies further confirmed that the pectin nanoparticles with galactose residues generated higher cytotoxicity than free honokiol on HepG2 cells which highly expressed asialoglycoprotein receptors (ASGR). Nevertheless, these findings were not observed in ASGR-negative A549 cells under similar condition. Therefore, pectin nanoparticles demonstrated a specific active targeting ability to ASGR-positive HepG2 cells and could be used as a potential drug carrier for treatment of liver-related tumors.

1. Introduction

Honokiol (HK), a lignanoid extracted from *Magnolia officinalis* or other species of *Magnoliaceae*, can inhibit the transfer and proliferation, promote differentiation, and induce apoptosis of tumor cells, as well as inhibit tumor angiogenesis (Deng et al., 2008; Hahm, Karlsson, Bonner, Arbiser, & Singh, 2014; Liang et al., 2010; Singh, Prasad, & Katiyar, 2013). Nevertheless, the poor water solubility of HK limits its clinical application. To improve the water solubility and bioavailability of HK, scholars have investigated several drug delivery systems, including microparticles (Li et al., 2009), nanoparticle (Zheng et al., 2010) and inclusion complex (Xu et al., 2014).

Targeted drug delivery systems can improve anti-tumor efficacy and reduce systemic toxicity by limiting the bioactivity of anticancer drugs to the localized tumor, as well as significantly improve therapeutic efficiency (Chen et al., 2013; Du et al., 2013; Huang et al., 2010; Paranjpe et al., 2004; Qu, Lin, Zhang, Xue, & Zhang, 2013); this system is dependent on passive nanoparticle capture through enhanced permeability and retention effect (Fang, Nakamura, & Maeda, 2011; Li et al., 2013; Torchilin, 2011) or active targeting based on overexpressed receptor recognition by binding to decorated ligands on the surface of carriers (Choi et al., 2010; Danhier, Feron, & Preat, 2010; Kolhatkar, Lote, & Khambati, 2011; Vhora et al., 2014). Asialoglycoprotein receptors (ASGR), which are mainly expressed on the surface of hepatocellular carcinoma cells, can be specifically recognized by galactose residues; the targeting efficiency of vehicles modified by galactose residues can be significantly enhanced by receptor-mediated endocytosis (Yik, Saxena, Weigel, & Weigel, 2002). As a result of the abundant binding sites on the cell surface and the high affinity between ligands and receptors, delivery systems with galactose residues can efficiently transport drugs to hepatocellular carcinoma cells (Diaz, Vargas, & Gatjens-Boniche, 2006; Ng, Chiang, Lin, & Lin, 2006).

Natural biodegradable polysaccharides have received increasing attention in drug delivery systems because of their nontoxicity, biocompatibility, biodegradability, and controlled drug release

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properties (Bagre, Jain, & Jain, 2013; Huang et al., 2013; Liu et al., 2012; Zu, Zhao, Zhao, Zu, & Meng, 2011). Pectin, an important polysaccharide, mainly consists of α-(1-4)-linked D-polygalacturonic acid residues (Cheng & Lim, 2004; Fan et al., 2008; Yu et al., 2009). Nanoparticles containing pectin may target hepatocellular carcinoma cells (Chittasupho, Jaturanpinyo, & Mangmool, 2013; Verma & Kumar, 2013; Yu et al., 2014). Nevertheless, pectin nanoparticles are hydrophilic gel nanoparticles and can efficiently encapsulate water-soluble drugs. However, water-insoluble drugs, such as HK, cannot be effectively incorporated into pectin nanoparticles in pure aqueous solutions.

In this study, hydroxypropyl-β-cyclodextrin (HCD) was used to fabricate an inclusion complex with HK (HKHCD) to increase the solubility of the drug and thus facilitate its encapsulation and dispersion in the pectin matrix. HKHCD was loaded onto pectin nanoparticles (HKHCD-PN) under mild condition without using any surfactant and organic solvent. The in vitro pharmaceutical properties of HKHCD-PN, such as encapsulation efficiency, drug loading content, particle size, particle size distribution, morphology, differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FT-IR), and in vitro drug release, were evaluated. In vitro cytotoxicity, cell apoptosis, cellular uptake, and targeting effect were further assessed in human liver hepatocellular carcinoma (HepG2) and human alveolar adenocarcinoma (A549) cells.

2. Materials and methods

2.1. Materials

Honokiol (HK, purity ≥98%) was obtained from Xi'an Xiaocao Botanical Development Co., Ltd. (Xi'an, China). Hydroxypropyl-β-cyclodextrin (HCD, DS = 4.2) was supplied by Xi'an Deli Biological Chemical Co., Ltd. (Xi'an, China). Pectin (galacturonic acid ≥65.0%) was provided by Quzhou Pectin Co., Ltd., (Quzhou, China). Dulbecco's Modified Eagle's Medium (DMEM)/High Glucose and Trypsin Solution (0.25%) were purchased from Thermo Fisher Scientific Biological Chemical Co., Ltd., (Beijing, China). Penicillin–Streptomycin Solution was obtained from Beijing Dingguo Changsheng Biotechnology Co., Ltd., (Beijing, China). MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-*H*-tetrazolium bromide) and Dimethyl Sulfoxide (DMSO) were supplied by Biosharp Biotechnology Co., Ltd. (Hefei, China). All the other reagents were of analytical grade.

2.2. Cell culture

HepG2 and A549 cells were provided by the Chongqing Key Laboratory of Biochemistry and Molecular Pharmacology (Chongqing, China). The cells were maintained in DMEM supplemented with 10% (v/v) new bovine serum and 1% (v/v) penicillin–streptomycin solution at 37 °C in a saturated humidity atmosphere containing 5% CO_2/95% air.

2.3. Preparation of HKHCD-PN

HK and HCD were mixed at a ratio of 1:3 (M/M) in 5 mL of distilled water under magnetic stirring (300 rpm) and then immersed in a thermostatic water bath at 60 °C for 30 min to obtain HKHCD. Pectin (0.10 g) was added in the HKHCD solution with stirring (300 rpm) for 30 min at 60 °C. Calcium hydroxide solution (0.01 M, 1 mL) was gradually added in the pectin solution with stirring (300 rpm) for 1 h at 60 °C. Sodium bicarbonate solution (0.01 M, 3 mL) was then added into the system dropwise with stirring (300 rpm) for 3 h at 60 °C. The system was rapidly cooled in a water bath at 20 °C for 30 min. The mixture (1 mL) was transferred in a dialysis bag and dialyzed against 150 mL of distilled water at 20 °C for 20 h to obtain HKHCD-PN. The sample was freeze-dried (ALPHA 1-2 LD plus, CHRIST, Germany) for 48 h to obtain dried HKHCD-PN and 5% (w/v) sucrose was used as cryoprotectant.

2.4. Characterization of HKHCD-PN

After HKHCD-PN dialysis, the drug loss reflected by the drug content in the aqueous phase outside the dialysis bag was determined through the absorbance at 292 nm by using a UV–vis spectrophotometer (UV-2600 SHIMADZU, Japan). Encapsulation efficiency and drug loading content were then calculated. Data were expressed as means of three independent measurements.

$$\text{encapsulation efficiency}(\%) = \frac{(\text{total amount of drug} - \text{drug loss})}{\text{total amount of drug}} \times 100$$

$$\text{drug loading content}(\%) = \frac{\text{drug recovered in nanoparticle}}{\text{nanoparticle recovered}} \times 100$$

The particle size and particle size distribution of HKHCD-PN in aqueous solutions were measured through laser light scattering at 25 °C with a Malvern Zetasizer (Nano ZS90, Malvern Instruments, UK).

The morphology of HKHCD-PN was observed by transmission electron microscope (TEM). A drop of HKHCD-PN was placed onto a carbon-coated copper grid. After 1 min, the excess staining solution was removed by touching the grid edge using a filter paper. The sample was naturally dried at room temperature. The naturally dried sample was then examined using transmission electron microscope (Hitachi-7500, Japan).

DSC was used to analyze the thermal characteristics of the powdered samples of HK, HCD, pectin, HKHCD, physical mixture, blank nanoparticles, and HKHCD-PN. Analyses were performed in a Netzsch STA449 C thermal analyzer (Netzsch Corporation, Germany). Three milligrams of sample was placed into the aluminum pan covered and fixed on the sample platform. Another empty hermetically sealed aluminum pan was used as reference to obtain the baseline. All samples were heated from 50 °C to 300 °C at a constant rate of 10 °C/min in an air speed of 40 mL/min nitrogen atmosphere.

The structure of pectin, HK, HCD, HKHCD, physical mixture, blank nanoparticle and HKHCD-PN were analyzed with an FT-IR spectrophotometer (Nicolet iS50 FT-IR, Nicolet Instruments, USA). All samples were mixed thoroughly with KBr in an agate mortar and then pressed into pellets. FT-IR spectra of the pellets were measured from 4000 cm^{-1} to 400 cm^{-1} at room temperature.

2.5. In vitro release studies

In vitro drug release studies were evaluated using dialysis method. HK, HKHCD, and HKHCD-PN (all samples contain 4.5 mg of HK) was placed into a pre-treated dialysis bag (molecular weight cutoff is 8–14 kDa). The bag was immersed in 150 mL of phosphate buffer solutions (PBS) with pH of 7.4 and constantly shaken (100 rpm) in a water bath (SHZ-88, Jiangshu Taichang Co., Ltd., China) at 37 ± 0.5 °C. At predetermined time intervals, the release medium outside the dialysis bag (4.0 mL) was removed and replaced with prewarmed fresh release medium. Drug concentration was determined through the absorbance at 292 nm by using a UV–vis spectrophotometer (UV-2600 SHIMADZU, Japan). Data were expressed as means of three independent measurements.