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NANOMEDICINE

Development of novel self-assembled DS-PLGA hybrid nanoparticles for improving oral bioavailability of vincristine sulfate by P-gp inhibition

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ABSTRACT

To improve the encapsulation efficiency and oral bioavailability of vincristine sulfate (VCR), novel self-assembled dextran sulphate-PLGA hybrid nanoparticles (DPNs) were successfully developed using self-assembly and nanoprecipitation method. By introducing the negative polymer of dextran sulphate sodium (DS), VCR was highly encapsulated (encapsulation efficiency up to 93.6%) into DPNs by forming electrostatic complex. *In vitro* release of VCR solution (VCR-Sol) and VCR-loaded DPNs (VCR-DPNs) in pH 7.4 PBS showed that about 80.4% of VCR released from VCR-DPNs after 96 h and burst release was effectively reduced, indicating pronounced sustained-release characteristics. *In vivo* pharmacokinetics in rats after oral administration of VCR-Sol and VCR-DPNs indicated that the apparent bioavailability of VCR-DPNs was increased to approximate 3.3-fold compared to that of VCR-Sol. The cellular uptake experiments were conducted by quantitative assay of VCR cellular accumulation and fluorescence microscopy imaging of fluorescent labeled DPNs in two human breast cancer cells including MCF-7 and P-glycoprotein over-expressing MCF-7/Adr cells. The relative cellular uptake of VCR-DPNs was 12.4-fold higher than that of VCR-Sol in MCF-7/Adr cells implying that P-glycoprotein-mediated drug efflux was diminished by the introduction of DPNs. The new DPNs might provide an effective strategy for oral delivery of VCR with improved encapsulation efficiency and oral bioavailability.

1. Introduction

Oral chemotherapy is a preferred alternative strategy in the cancer treatment due to its convenience, patient compliance, and cost-effectiveness. Unfortunately, the majority of anticancer drugs have a low and variable oral bioavailability [1], which greatly limits the progress of oral cancer chemotherapy. Enhancement of bioavailability of cytotoxic agents is a pre-requisite for successful development of oral modes of cancer treatment.

Vincristine sulfate (VCR, Fig. 1A), as an effective chemotherapeutic agent, has been used extensively for treatment of various cancers [2]. However, over-expression of P-glycoprotein (P-gp) is one of the major obstacles to successful cancer therapy with VCR [3]. VCR are seldom administered by oral route because of the fast elimination and low absorption resulted by high P-gp-mediated efflux. The shortcomings of VCR may be tackled by incorporation into drug delivery systems.

As drug carriers, PLGA nanoparticles possess moderate P-gp inhibition effect and MDR reversal activity on their own [4]. The incorporation of antitumor drugs into PLGA nanoparticles would be a possible approach to improve oral bioavailability. So far many lipophilic drugs have been encapsulated easily into PLGA nanoparticles. However, encapsulation of low molecular weight water-soluble ionic drugs, such as vincristine sulfate, may be a great challenge for PLGA nanoparticles using the common preparation methods. Many strategies have been utilized to enhance the encapsulation of water-soluble drugs into PLGA hydrophobic matrix, such as nanoprecipitation [5], O/W single emulsification [6,7], W/O single emulsification [8] and W/O/W double emulsification [9,10]. However, some of the formulation processes were relatively complicated and others had slightly improved entrapment efficiency.

In order to enhance the encapsulation efficiency and oral bioavailability of VCR, we attempted to develop novel dextran sulphate-PLGA hybrid nanoparticles (DPNs) by nanoprecipitation and self-assembly method in this study. The prepared DPNs were composed of PEG shell, lecithin monolayer, and DS-PLGA hybrid core, which integrated the structures of liposomes and biodegradable polymeric nanoparticles.

In our design, dextran sulphate sodium (DS, Fig. 1B) as a counter ion polymer was expected to form complex with cationic VCR, and then the charge neutralization would allow the instantaneous entrapment of the complex into PLGA. The prepared DPNs were characterized by particle size, Zeta potential, encapsulation efficiency, transmission electron microscopy, and differential scanning calorimetry. The effects of electrostatic interaction between VCR and DS on drug loading and release profile were investigated by comparing the

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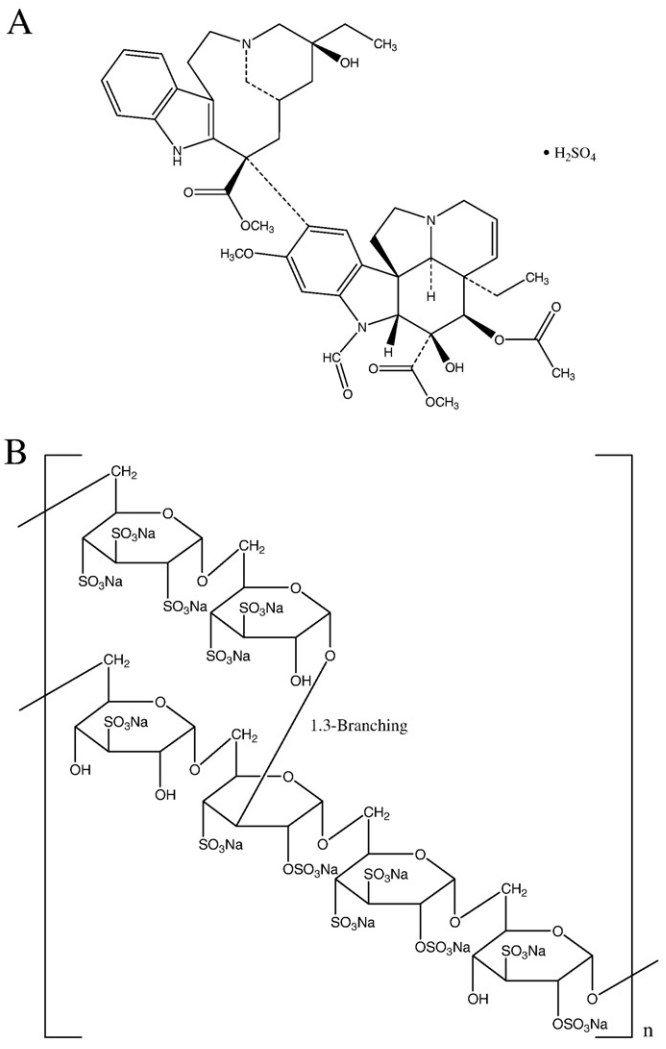

Fig. 1. Chemical structures of (A) vincristine sulfate and (B) dextran sulfate sodium.

in vitro characteristics of DPNs containing different ratios of drug-polymer. The oral pharmacokinetics and bioavailability of VCR-loaded DPNs (VCR-DPNs) and VCR solution (VCR-Sol) in rats were conducted to investigate the absorption enhancement of DPNs. To clarify whether the DPNs had P-gp inhibition effect, the cellular uptake experiments were carried out in MCF-7 cells and P-gp over-expressing MCF-7/Adr cells. The effect of DPNs on the VCR intracellular accumulation in MCF-7/Adr cell was compared with the P-gp inhibitor verapamil (VRP). Meanwhile, the fluorescence microscopy images of cellular uptake of rhodamine 123-labeled DPNs (R123-DPNs) in MCF-7/Adr cell were visualized to further confirm the P-gp inhibition effect of DPNs.

To our best knowledge, this is the first report that an anionic polymer DS is introduced into PLGA nanoparticles to increase encapsulation efficiency of water-soluble cytotoxic drug and applied for oral chemotherapy. The systematic studies attempt to provide more insights that the DPNs might act as a prospective carrier to improve the gastrointestinal absorption and oral bioavailability of encapsulated cytotoxic drugs.

2. Materials and methods

2.1. Materials

Vincristine sulfate (VCR, 98.9% purity) was provided by Shanghai Anticancer Phytochemistry Tech. Co., Ltd. (Shanghai, China). Poly(DL-lactic-co-glycolic acid) (PLGA, 75:25, Mw 10,000) was obtained from Shandong Institute of Medical Instrument (Jinan, China). 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (PEG–PE) was purchased from Avanti Polar Lipids (Alabaster, AL). Dextran sulfate sodium (DS, Mw 5,000) was supplied by Shanghai Xibao Biotech. Co., Ltd. Lecithin was purchased from BASF (D-Ludwigshafen, Germany). Trypsin, RPMI 1640 medium and fetal bovine serum (FBS) were purchased from Gibco BRL, USA. Rhodamine 123 (R123) and others materials were supplied by Sigma (Germany). All solvents used in this study were of HPLC grade.

2.2. Preparation and characterization of VCR-DPNs

2.2.1. Preparation of VCR-DPNs

VCR-DPNs were prepared by a combined self-assembly technique and nanoprecipitation method as previously described with some modifications [11,12]. In brief, PLGA polymer was dissolved in acetonitrile with concentration of 25 mg/ml. Lecithin/PEG-PE (8.8/1.2, molar ratio) with a weight ratio of 15% to the PLGA polymer were dispersed in 4% ethanol aqueous solution at 65 °C. Then the VCR (with a weight ratio of 12.5% to PLGA) and DS (with different charge ratio of 1:0.5 and 1:1 to VCR) aqueous solutions were added by drops into the lecithin/PEG-PE solution under magnetic stirring, respectively. After 5 min of gentle stirring of the combined solution, the PLGA solution was injected dropwise. The mixed solution was vortexed vigorously for 3 min followed by gentle stirring for 2 h at room temperature. The remaining organic solvents were removed under reduced pressure at 37 °C. After metered volume by distilled water, the final concentration of VCR in nanoparticle suspensions was 0.5 mg/ml. To confirm the effect of DS on the enhancement of encapsulation efficiency, the PLGA nanoparticles without DS (PLGANPs) were prepared as the same procedure except that the DS solution was replaced by distilled water. The blank PLGANPs were also prepared as the same procedure without adding VCR and DS.

2.2.2. Particle size and Zeta potential

The particle size of the prepared nanoparticles was determined using a Coulter LS 230 laser diffraction instrument (Beckmann-Coulter Electronics, Krefeld, Germany). The nanoparticle suspensions were diluted 30 folds with water for injection to give an intensity of 300 Flux as recommended by the manufacturer. The Zeta potential was measured by a Zeta potential analyzer (Delsa 440SX, Beckmann-Coulter Electronics, Germany) with the nanoparticles diluted in water. All the analyses were carried out in triplicate.

2.2.3. Drug encapsulation efficiency

The encapsulation efficiency of VCR in nanoparticles was determined applying Sephadex G50 micro column centrifugation method. To separate the loaded VCR in nanoparticles and free VCR, 0.5 ml nanoparticle suspension in water with VCR concentration of 0.5 mg/ml was loaded into the Sephadex micro column and eluted by 1 ml distilled water for several times through centrifugation at 1000 r/min. The eluted fractions containing VCR-loaded nanoparticles and free VCR were collected respectively for determination of VCR by HPLC-UV at 297 nm.

For the determination of loaded VCR in nanoparticles, 1 ml nanoparticle suspension and suitable quantity of acetonitrile/0.1 M HCl (3:1, v/v) were mixed by sonication for 10 min to destroy the nanoparticles and then the volume was metered to 25 ml. After filtration by 0.45 μm filter membrane, an aliquot of 20 μl filtrate was analyzed by a validated HPLC-UV method for determination of VCR concentration. Chromatographic separation was carried out on a Diamonsil C_{18} column (200 mm × 4.6 mm, 5 μm; Dikma Technologies, China) using methanol–ammonium acetate (5 mM)–acetic acid (60:40:0.1, v/v/v) as mobile phase at detection wavelength of 297 nm.