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Novel hydrophobin-coated docetaxel nanoparticles for intravenous delivery: In vitro characteristics and in vivo performance

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ABSTRACT

Novel hydrophobin (H star Protein® B, HPB)-coated docetaxel (DTX) nanoparticles were designed for intravenous delivery. DTX-HPB nanoparticles (DTX-HPB-NPs) were prepared using a nanoprecipitation–ultrasonication technique. The physicochemical properties in terms of particle size, size distribution, zeta potential, morphology, crystalline state of the drug, in vitro release and plasma stability were evaluated. To investigate the drug–hydrophobin interaction, FTIR analysis was carried out. The pharmacokinetics of DTX-HPB-NPs and Taxotere were compared after i.v. administration to rats. The optimized formulations have a high drug loading (>25%) and nanoparticle yield (>93%), small particle size with a narrow distribution, and exhibit delayed release. X-ray diffraction (XRD) demonstrated that the drug is present in a crystalline state. FTIR analysis suggested that the interaction of DTX and HPB involved hydrogen bonding. In vitro hemolysis study confirmed the safety of these nanoparticles. In plasma, DTX-HPB nanoparticles exhibited a significantly enhanced C_{max} (1300.618 ± 405.045 ng/mL vs 453.174 ± 164.437 ng/mL, $p < 0.05$), and AUC_{0-t} (409.602 ± 70.267 vs 314.924 ± 57.426 μg/L h, $p < 0.05$), and a significantly reduced volume of distribution (36.635 ± 15.189 vs 95.199 ± 40.972 L/kg, $p < 0.05$) compared with the Taxotere. These results demonstrated that hydrophobin has the potential to be used as a novel biocompatible biomaterial for drug delivery.

1. Introduction

Over the last few decades, an increasing number of newly developed drugs have been found to be poorly water-soluble and, as a result, these so called 'brickdust drugs' often exhibit poor bioavailability (Porter et al., 2007; Rabinow, 2004). Different methods and formulations have been developed to overcome this problem and there has been considerable interest in the development of novel drug delivery systems using nanotechnology. For example, polymeric nanocarriers (Ernsting et al., 2012; Jones and Leroux, 1999) and lipid nanocarriers (Liu et al., 2011; Lowery et al., 2011) are now widely used. Numerous synthetic and natural polymers have been extensively investigated as polymeric materials for drug delivery applications. In any case, to be considered as a suitable material to deliver drugs in vivo, a polymer needs to fulfill several basic requirements (Nair and Laurencin, 2007). Firstly, it should be biocompatible and any potential degradation products should not have toxic or immunogenic effects. Secondly, the material should still have an acceptable long-term stability and it should possible to carry out some processing procedures during the preparation. Natural polymers, such as natural polysaccharides and their derivatives (Ernsting et al., 2012; Liu et al., 2008), gelatin (Lu et al., 2004), albumin (Han et al., 2010; Sebak et al., 2010) are described in detail in the literature for the preparation of drug delivery systems.

Biosurfactants are an alternative to common natural polymers, are produced by microorganisms, and have pronounced surface and emulsifying activities due to their amphiphilicity (Mulligan, 2005). Hydrophobins are a family of low molecular weight proteins with a characteristic pattern of cysteine residues that form four disulfide bonds, and are exclusively found in fungi. They self-assemble to form robust polymeric monolayer films that are highly amphipathic and play an important role in fungal growth and development, forming protective films and their action is mediated by the attachment of fungi to solid surfaces (Hektor and Scholtmeijer, 2005; Sunde et al., 2008). Secreted hydrophobins have the ability to convert hydrophobic surfaces to hydrophilic ones and hydrophilic surfaces to hydrophobic ones by self-assembling into

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an amphipathic protein membrane. On the basis of differences in hydropathy patterns and aqueous solubility of their assembled films, hydrophobins are classified into Class I and Class II hydrophobins (Linder et al., 2005). Hydrophobins, as fungal protein have attracted more attention in a number of pharmaceutical fields. Class I hydrophobin SC3 has been used to increase oral drug bioavailability (Haas Jimoh Akanbi et al., 2010), and Class II hydrophobin HFB II-coated porous silicon nanoparticles have been used to improve the biocompatibility and change the biodistribution after intravenous injection (Sarparanta et al., 2012). In addition, HFB II can be genetically modified to bioengineer nanoparticles, offering special bindings domain for target adhesion (Valo et al., 2011). Hydrophobins generally are considered to be safe because they are present in button mushrooms and other edible fungi (Paslay et al., 2013). As far as we know, to date, no reports have described whether in vivo pharmacokinetics can be modified by hydrophobin-coated drug nanoparticles after intravenous administration.

Docetaxel (DTX), an analog of paclitaxel, is an effective and widely used anticancer drug in clinical practice (Rowinsky, 1997). However due to its high lipophilicity and low solubility, the main commercial formulation (Taxotere®) that is used clinically is formulated in Tween 80 and ethanol, which requires dilution in normal saline or 5% dextrose solution prior to intravenous administration. Tween 80 carries a high risk of producing serious side effects in patients, such as allergic reactions, neurotoxicity, and nephrotoxicity (Clarke and Rivory, 1999). Therefore, the development of an alternative drug delivery system for DTX is urgently required.

In this work we describe the design and developed hydrophobin-coated drug nanoparticle to investigate the in vivo pharmacokinetic characteristics after intravenous administration, using DTX as a model hydrophobic drug. DTX-HPB nanoparticles were prepared by a nanoprecipitation–ultrasonication technique, and the physicochemical properties were characterized. The particle size distribution was determined by dynamic light scattering (DLS) and the morphology of the nanoparticles was characterized by transmission electron microscopy (TEM). The drug state in the nanoparticles was characterized by X-ray diffraction (XRD) and the drug–hydrophobin interaction was investigated by Fourier transform infrared spectroscopy (FTIR). An in vitro drug release study was conducted using an equilibrium dialysis method to evaluate the influence of hydrophobin on the release behavior of DTX. Finally, hemolysis test was carried out and the in vivo pharmacokinetics of DTX-HPB nanoparticles was evaluated to demonstrate that hydrophobin-coated drug nanoparticles can be used as an intravenous drug delivery system for hydrophobic drug.

2. Materials and methods

2.1. Materials and animals

Docetaxel and paclitaxel were purchased from (Shanghai sanwei Pharma Ltd. Co., Shanghai, China), H Star Protein® B, from now on abbreviated as HPB (18.8 kDa; IEP: 5.9; purity: 96%), is recombinant hydrophobins and is a gift from BASF, Ludwigshafen (Germany), and it belongs to Class I hydrophobins. While tert-butyl methyl ether (TBME, Sinopharm Chemical Reagent Ltd. Co., Shenyang, China), formic acid (Dima Technology Inc., Richmond Hill, USA), methanol, acetonitrile and dehydrated alcohol (Tianjin Concord Technology Ltd., Co., Tianjin, China) were obtained from the sources indicated, all other chemicals and reagents were of analytical or chromatographic grade.

Male Sprague–Dawley rats (200 ± 20 g) were obtained from the Experimental Animal Center of Shenyang Pharmaceutical University and housed at 22 ± 2 °C with access to food and water ad libitum. The protocol for the animal experiments was approved by the Shenyang Pharmaceutical University Institutional Animal Care and Use Committee.

2.2. Preparation of hydrophobin solution

Prior to use, to ensure complete dissolution, a weighed amount of hydrophobin was dissolved in pure deionized water with magnetic stirring for about 2 days at room temperature. Then, the protein solution was passed through a 0.8 μm filter membrane to remove impurities in the protein solution. Finally, a clear protein solution was obtained which was used in all subsequent studies.

2.3. Preparation of hydrophobin-coated drug nanoparticles and bare drug nanoparticles

A nanoprecipitation–ultrasonication technique (He et al., 2013) was used to prepare the nanoparticles. Methanol was used as a solvent for DTX and HPB aqueous solution was obtained as described above. Then, 0.5 mL of DTX solution was added dropwise to 10 mL of HPB aqueous solution. The resulting mixed solution was ultrasonicated using probe sonication (Sonics & Material Vibra Cell, 750 W, 20 kHz) at 53% amplification (400 W) for 10 min with a 3 s pulse-on period and a 3 s pulse-off period in an ice bath to keep the temperature low. The methanol was removed by rotary vacuum evaporation and then the nanoparticle suspension was centrifuged (8000 rpm, 10 min) and passed through a 0.8 μm cellulose ester filter membrane to remove the uncoated drug and any impurities. Bare drug nanoparticles were prepared as for the hydrophobin-coated nanoparticles except that no hydrophobin was present.

2.4. Particle size and zeta potential measurements

The average particle size, size distribution, and zeta potential of the nanoparticles were determined by dynamic light scattering (PSS NICOMP 380, USA) at room temperature. He–Ne was used as light source, a laser beam at a wavelength of 632.8 nm, and the scattering angle was set at 90° when particle size measurements were conducted. Zeta potential measurements were performed at a laser beam wavelength 633 nm and a scattering angle 18.9°. Prior to making these measurements, all samples were diluted with deionized water to a suitable concentration, avoiding any multiscattering. Each parameter was measured three times, and the average values and standard deviations were calculated.

2.5. Determination of hydrophobin nanoparticle yield

In order to determine the amount of HPB transformed into nanoparticles, the hydrophobin-coated DTX nanoparticles were separated from the supernatant by centrifugation at 50,000 rpm for 2 h at 4 °C using a high speed refrigerated centrifuge (HITACH, Japan). An aliquot of the supernatant was diluted with deionized water and the amount of the HPB in the supernatant was determined using a standard Coomassie Brilliant Blue protein assay (Sedmak and Grossberg, 1977). The calculations were made on the base of standard curve which was $r > 0.996$ in the concentration range 20–80 μg/mL. All sample analyses were carried out in triplicate. The yield of nanoparticles (Y_{np}) could be calculated as the follow equation:

$$Y_{np}\ (\%) = \frac{W_{totalHPB} - W_{freeHPB}}{W_{totalHPB}} \times 100$$

2.6. Particle morphology

The morphology of the hydrophobin-coated docetaxel nanoparticles was visualized by transmission electron microscopy (TEM)