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Hydrophobically modified inulin as an amphiphilic carbohydrate polymer for micellar delivery of paclitaxel for intravenous route

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ABSTRACT

Micellization offers several advantages for the delivery of water insoluble drugs including a nanoparticulate 'core–shell' delivery system for drug targeting. Recently, hydrophobically modified polysaccharides (HMPs) are gaining recognition as micelle forming polymers to encapsulate hydrophobic drugs. In this manuscript, for the first time, we have evaluated the self-assembling properties of a lauryl carbamate derivative of the poly-fructose natural polymer inulin (Inutec SP1® (INT)) to form paclitaxel (PTX) loaded micelles. INT self-assembled into well-defined micellar structures in aqueous environment with a low critical micellar concentration of 27.8 μg/ml. INT micelles exhibited excellent hemocompatibility and low toxicity to cultured cells. PTX loaded INT micelles exhibited a mean size of 256.37 ± 10.45 nm with excellent drug encapsulation efficiency (95.66 ± 2.25%) and loading (8.69 ± 0.22%). PTX loaded micelles also displayed sustained release of PTX and enhanced anti-cancer efficacy *in-vitro* in mouse melanoma cells ($B_{16}F_{10}$) compared to Taxol formulation with Cremophor EL as solvent. In addition, PTX loaded INT micelles exhibited comparable *in-vivo* antitumor activity in $B_{16}F_{10}$ allograft mouse model at half the dose of Taxol. In conclusion, INT offers safe, inexpensive and natural alternative to widely used PEG-modified polymers for the formulation of micellar delivery systems for paclitaxel.

1. Introduction

Paclitaxel (PTX) is a potent natural anticancer drug effective in treating a wide array of cancers including breast cancer, skin cancer, ovarian cancer, stomach cancer, non-small cell lung cancer, AIDS related Kaposi's sarcoma *etc.* (Singla et al., 2002). Poor aqueous solubility of PTX (<2 μg/ml) (Liggins et al., 1997) is one of the toughest challenges in utilizing its full clinical potential. Solubility enhancement by producing alternate salts or addition of charged complexing agents is not feasible approach for PTX (Sharma et al., 1995) because of lack of polarity. As a result, PTX is currently solubilized in a 50:50 mixture of Cremophor EL® and dehydrated ethanol and marketed as Taxol® (Liang et al., 2012). However, Cremophor EL® has been associated with severe anaphylactic reactions, hyperlipidemia, aggregation of erythrocytes, hemolysis, and peripheral neuropathy (Gelderblom et al., 2001). Thus, there is a need for better-tolerated and less toxic carriers for PTX delivery.

Micellization offers a unique advantage of improving the solubility of poorly water-soluble drugs and at the same time provides a nanoparticulate 'core–shell' delivery system. This distinctive core–shell architecture enables the micelles to effectively solubilize poorly water-soluble drugs in the core by hydrophobic interactions and still maintain hydrophilicity of the shell (Kwon and Okano 1996). Such hydrophilic polymeric micelles have been shown to reduce non-selective reticuloendothelial system (RES) scavenging and improve circulation time (Kataoka et al., 2000). Also, the nano-scaled dimensions of polymeric micelles enhance tumor accumulation via the enhanced permeability and retention (EPR) effect (Maeda et al., 2000).

A plethora of polymers have been investigated in the past for micelle formation. Adjustments of chemical nature and length of

Abbreviations: PTX, paclitaxel; RES, reticulo-endothelial System; EPR, enhanced permeability and retention; PEG, poly-ethylene glycol; ABC, accelerated blood clarence; HMP, hydrophobically modified polysaccharides; INT, Inutec SP1®; CMC, critical micellar concentration; NMR, nuclear magnetic resonance; TEM, transmission electron microscopy; DLS, dynamic light scattering; HPLC, high performance liquid chromatography; EE, entrapment efficiency; DL, drug loading.

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polymer component, the hydrophilic/hydrophobic balance, and conditions of self-assembly, have led to characterization of polymeric micelles with well-defined architecture for improving therapeutic efficacy (Wang et al., 2012a; Shuai et al., 2004). The hydrophilic component of a broad variety of these amphiphilic polymers is composed of polyethylene glycol (PEG), mostly due to its low-toxicity, low immunogenicity and a tightly bound water layer (Zhang et al., 2012). However, recent reports suggests several unfavorable biologic responses following PEGylation of nanocarriers such as polymeric micelles and liposomes. PEGylated micelles more than 50 nm have been shown to induce a phenomenon termed accelerated blood clearance (ABC), similar to PEGylated liposomes (Koide et al., 2008). Several research groups have clearly shown that a repeated injection of PEGylated nanocarriers including micelles results in the production of anti-PEG IgM antibodies from splenic B-cells. Anti-PEG IgM produced in response, binds selectively to the subsequently injected PEGylated nanoparticulate system and results in their rapid clearance (Koide et al., 2008; Shiraishi et al., 2013). In addition, PEGylation may potentially interfere with binding and uptake in the target cells (Hatakeyama et al., 2011). The quest for alternative hydrophilic components for micelle forming polymers, without the caveats of PEGylation, has led to the hydrophobic modification of polysaccharides. Hydrophobically modified polysaccharides (HMPs) have been shown to self-assemble forming micellar nanoparticulate systems (Mahmoudzadeh et al., 2013; Na et al., 2003; Nichifor et al., 2014; Zhang et al., 2013). Such systems are unique in having extremely hydrophilic polysaccharide backbone as shell and a hydrophobic core to encapsulate poorly water soluble drugs and also act as an effective carrier for delivery of such hydrophobic drugs. (Nishikawa et al., 1996; Wu et al., 2014).

The lauryl carbamate derivative of inulin, Inutec SP1® (INT) offers excellent tensioactive properties and hence has found a use as an emulsifier in pharmaceutical industry even in presence of high electrolyte content (Van den Mooter et al., 2006). INT has also been utilized as a polymer for formulation of solid dispersions to improve solubility of poorly water soluble drugs (Van den Mooter et al., 2006). Although INT possesses excellent physicochemical characteristics such as desirable hydrophobic/hydrophilic balance (HLB = 8), and excellent tensioactive properties, it has not been explored yet for micelle formation.

In this study for the first time, the ability of INT is evaluated to form self-aggregated micelles. INT micelles were utilized to encapsulate PTX. The resulting INT-PTX micelles were characterized in terms of their physicochemical characteristics including size, ζ-potential, drug loading and critical micellar concentration (CMC). *In-vitro* cytotoxic effect was studied on mouse melanoma cells ($B_{16}F_{10}$). The *in-vivo* efficacy was performed in an allograft mouse model using subcutaneous $B_{16}F_{10}$ tumors in C57BL/6J mice.

2. Materials and methods

2.1. Materials

Inutec SP1® (CAS number 478483-27-1) was kindly gifted by Beneo bio-based chemicals, Wijgmaal, Belgium. Paclitaxel (CAS number 33069-62-4) was purchased from LC laboratories, Woburn MA, USA. Pyrene (CAS number 129-00-0), Methanol (CAS number 67-56-1), Acetonitrile (CAS number 75-05-8), Dimethyl Sulfoxide (DMSO) (CAS number (67-68-5)), 3-[4,5-dimethylthiazolyl-2]-2,5-diphenyl tetrazolium bromide (MTT) (CAS number 298-93-1) were purchased from Sigma–Aldrich, St. Louis, MO, USA. Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), trypsin–EDTA and penicillin–streptomycin was purchased from Fisher Scientific, Pittsburgh, PA, USA. Mouse melanoma cell line $B_{16}F_{10}$ (CRL-6475) was purchased from ATCC, Manassas, VA, USA. C57BL/6J mice were obtained from Charles River laboratories Chicago, IL, USA. Procedures involving animals complied with ethical guidelines and were approved by the South Dakota State University Institutional Animal Care and Use Committee (IACUC).

2.2. High-resolution proton nuclear magnetic resonance (^{1}H-NMR) analysis of INT

The chemical characterization and the presence of carbamate function in INT was confirmed by high-resolution proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy using a Bruker 400 MHz NMR spectrometer (Bruker, Karlsruhe, Germany) operated at 400 MHz. Native Inulin and carbamate modified INT were dissolved in deuterated Dimethyl Sulfoxide (*d-6* DMSO) at a concentration of 1% (w/v) for NMR recording.

2.3. The critical micellar concentration (CMC) of INT

The capability of INT to form hydrophobic nano-domains was assessed by determination of CMC using pyrene as a fluorescent probe. Briefly, a solution of 1 mg of pyrene in acetone (6×10^{-6} M) was added in a series of glass vials and evaporated. Following this, 10 ml of deionized water containing various concentrations of INT from 0.0001 mg/ml to 10 mg/ml was added and the solution was incubated overnight under mild stirring. The fluorescence intensity of the resulting solutions was determined using a spectrofluorometer. The emission spectrum was determined at 375 nm (I_1) and 384 nm (I_3) while the excitation wavelength was fixed at 334 nm. The CMC was determined by taking the midpoint of the INT concentration at which the relative fluorescence intensity ratio of I_3/I_1 was varied (Daman et al., 2014; Liang et al., 2012).

2.4. Preparation of PTX loaded INT micelles

As a starting point, the PTX loaded INT micelles were prepared by using two different methods; thin film hydration and solvent evaporation. For both these techniques, the initial drug to polymer (*D*/*P*) ratio of 5% and 10% w/w was used to prepare micelle formulations. In the thin film hydration method (Daman et al., 2014), both PTX and polymer (50 mg) were dissolved in Tetrahydrofuran (THF) followed by vacuum drying (overnight) in a rotary evaporator BUCHI Rotary evaporator R II (BUCHI Corporation, DE, New Castle) and subsequent hydration with deionized water (MilliQ, Millipore) under vigorous stirring for 30 min at room temperature. The formed micelles were subjected to bath sonication for 5 min.

Micelles by solvent evaporation method were formulated using a probe-type ultrasonic method (Sonics Vibra Cell ultrasonic liquid processor) (Liang et al., 2012). Firstly, 50 mg of INT was dissolved in 10 ml of MilliQ water, and then PTX–acetone solution (1 mg/ml) was added into the aqueous phase under probe-sonication. The resultant mixture was further sonicated at 400 W for 30 min over an ice bath, with a pulse (3 s on and 2 s off) to avoid heating.

Four formulations were prepared with 5 and 10% *D*/*P* ratio by thin film hydration and solvent evaporation techniques respectively:

Formulation A: 5% *D*/*P* ratio; solvent evaporation method
Formulation B: 10% *D*/*P* ratio; solvent evaporation method
Formulation C: 5% *D*/*P* ratio; thin film hydration method
Formulation D: 10% *D*/*P* ratio; thin film hydration method

In order to remove the unincorporated PTX form formulations, the micelle suspensions were centrifuged at $3200 \times g$ for 15 min. The resultant supernatant containing the micelles was lyophilized by addition of mannitol (10% w/w) as a cryoprotectant using (Virtis SP Scientific Benchtop Lyophilizer) at a condenser temperature of