International Journal of Pharmaceutics 462 (2014) 1–7

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

A novel aqueous parenteral formulation of docetaxel using prodrugs

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ARTICLE INFO

Article history:
Received 17 September 2013
Received in revised form
28 November 2013
Accepted 15 December 2013
Available online 25 December 2013

Keywords:
Docetaxel
Prodrug
Parenteral
Solubility
Hemolysis

ABSTRACT

The aim of this study is to develop an aqueous parenteral solution of docetaxel using prodrugs. Docetaxel (DTX) is a highly lipophilic drug and practically insoluble in water. To overcome insolubility of docetaxel, three kinds of docetaxel prodrugs were synthesized using succinyl linker such as DTX-G, DTX-L or DTX-S and physicochemically characterized. The solubility of docetaxel prodrugs was determined by changing the concentration and type of surfactants, cosolvents or cyclodextrins. It was observed that the novel mixture of 15% PEG 400, 2.5% Tween 80 and 20% hydroxypropyl-β-cyclodextrin significantly increased the solubility of DTX-G up to 5.7 mg/mL. After subjected to the study of the hemolytic and cytotoxic activities, it was shown that the novel mixture did not show the hemolysis compared to Taxotere®. It was suggested this novel mixture might have the potential to develop an aqueous parenteral formulation.

1. Introduction

Prodrugs should be considered a chemistry-enabled drug delivery tool used to address shortcomings in the bioavailability, efficacy or safety profile of otherwise promising candidates. Prodrugs should be evaluated for enhancing compound solubility in addition to salts and co-crystals, as much as formulation-enabled drug delivery technologies (i.e. nanomilling, amorphous solid dispersions and other solubilizing vehicles) are considered routinely (Huttunen et al., 2011; Rautio et al., 2008).

Docetaxel (DTX) is an anticancer agent of the taxoid family. An analog of paclitaxel, DTX was obtained by semisynthesis from 10-deacetyl baccatin III extracted from the needles of the European yew tree, *Taxus baccata* L. (Denis et al., 1988; Mangatal et al., 1989) and is more effective than paclitaxel as an inhibitor of microtubule polymerization. DTX combined with certain other chemotherapeutic drugs shows high anticancer efficacy in breast, pancreatic, gastric, and urothelial carcinoma. Due to poor solubility of DTX, Taxotere® contains 40 mg/mL of anhydrous DTX and 1040 mg/mL of Tween 80, which is prepacked with a diluent vial containing a 13% (w/w) solution of ethanol in water. It is crucial to reconstitute the injection concentrate with the complete content of the diluent vial to ensure a concentration of 10 mg/mL DTX in the resulting premix solutions (Kraynak, 1997). Tween 80 frequently causes untoward hypersensitivity reactions such as hypotension, bronchospasm and urticaria. Also, it hampers the clinical usefulness because of its haemolyticus and viscidity, especially for the patients who are hypersensitive to Tween 80 (Weiss et al., 1990). In order to eliminate the Tween 80-based vehicle and to increase DTX solubility, alternative dosage forms have been suggested including liposomes (Immordino et al., 2003; Alexopoulos et al., 2004) as well as design of water-soluble prodrugs (Du et al., 2007), use of additives such as complexing agents (Grosse et al., 1998; He et al., 2003), co-solvents (Yalkowsky and Roseman, 1981), surfactants (Kawakami et al., 2004) or a combination effect of any of the above.

The aim of this study is to develop an aqueous parenteral formulation of DTX using prodrug, which was synthesized using glucose or sialic acid to improve the solubility of DTX. It was reported that the linkage between DTX and glucose or sialic acid was cleavage by esterase in the body fluid after administration in the form of prodrug, subsequently parent DTX exhibits its own pharmacological activities (Woo et al., 2011). The physicochemical characterizations of DTX prodrugs were carried out and the potential DTX prodrug was formulated for a parenteral formulation through this study.

2. Materials and methods

2.1. Materials

DTX was supplied from SamyangGenex (Daejeon, Korea). Propylene glycol (PG), glycerine and polyethylene glycol 400 (PEG 400)

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0378-5173/$ – see front matter.
http://dx.doi.org/10.1016/j.ijpharm.2013.12.027

were purchased from Duksan Pure Chemical Co. Ltd. (Ansan, Korea). Cyclodextrins such as α-cyclodextrin (α-CD), β-cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD) were obtained from Roquette Freres (Lestrem, France). Hydroxypropyl-β-cyclodextrin (HPCD) and Tween 80 were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ethanol was purchased from J.T. Baker (Phillipsburg, NJ). Poloxamer 188 (Lutrol® F 68), poloxamer 407 (Lutrol® F127) and cremophor EL (polyoxyl 35 castor oil) were purchased from BASF (Ludwigshafen, Germany). D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS), dimethyl sulfoxide (DMSO) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) was purchased from Sigma–Aldrich (St. Louis, MO, USA). All other solvents and chemical reagents were commercial products of analytical or reagent grade and were used without further purification.

2.2. Synthesis of DTX prodrugs

DTX prodrugs were supplied from Genechem (Daejeon, Korea). Briefly, 2-hydroxyl group of DTX was esterized using the succinyl linker and monosaccharide D-glucose, the disaccharides D-lactose or the amino carbohydrates sialic acid and DTX-G, DTX-L or DTX-S was synthesized and characterized Huttunen et al. (2011).

2.3. Cell cultures

HepG2 cells were purchased from the Korean cell line bank (Seoul, Korea). HepG2 cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% FBS, 100 units/mL penicillin and 0.1 mg/mL streptomycin in a 5% CO_2 atmosphere with 95% humidity in a 37 °C incubator.

2.4. HPLC assay

The concentration of DTX and DTX prodrugs was determined using an Agilent 1100 HPLC (Agilent Technologies, Palo Alto, CA) in which an phenomenax®C18 column (5 μm particle size, 4.6 mm × 250 mm) was used. The mobile phase was consisted of acetonitrile and distilled water in the ratio of 51:49 (v/v). The total run time was 15 min. The column temperature was maintained at 35 °C and the flow rate was 1.0 mL/min. UV absorption was measured at 230 nm. The sample preparation was also filtered through PVDF filter (0.45 μm).

2.5. Physicochemical characterization of DTX or DTX prodrugs

For observing the morphology of DTX or DTX prodrugs, samples were fixed on copper grid using conductive double-sided tape and then made electrically conductive by coating in a vacuum with a thin layer of gold. Then, they were examined for morphology with a FE-SEM (JEOL JSM7500, Thermo, USA) with 5 kV. In order to assess the change of solid state, differential scanning calorimeter (DSC) analysis was performed on DTX or DTX prodrugs. Accurately weighed samples of about 2 mg were analyzed in aluminum pans on a differential scanning calorimeter (DSC S 650, SCINCO, Korea). The DSC runs were conducted from 20 to 300 °C at a rate of 10 °C/min. The changes of crystallinity of DTX or DTX prodrugs were observed by X-ray powder diffraction (XRD). Data were collected over an angular range comprised between 5° and 70° with a step size of 0.01°.

2.6. Determination of partition coefficient of DTX or DTX prodrugs

The partition coefficients of DTX or DTX prodrugs were evaluated using water and 1-octanol. About 5.0 mg of DTX or DTX prodrugs was weighed accurately and dissolved in the same volume of water and 1-octanol. The samples were then shaken horizontally at 25 °C on a rotator (Barnstead Thermolyne, Sparks, NV) with 8 rpm for 3 h and then allowed to stand for another 30 min. The mixtures were then centrifuged for 15 min at 3000 rpm. The aqueous phases were analyzed before and after equilibration by HPLC analysis. The partition coefficient ($\log P$) was calculated from the equilibrium concentration of solute in 1-octanol divided by the concentration of the same species in water.

2.7. Solubility measurement of DTX or DTX prodrugs

The solubility of DTX or DTX prodrugs in aqueous was determined by the Higuchi and Connor methods (Higuchi and Connors, 1965). Excess of DTX or DTX prodrugs was added to 1 mL of various pH solutions. The samples were put on an end-to-end labquake rotator (Barnstead Thermolyne, Sparks, NV) at 8 rpm at ambient temperature for 72 h in order to achieve equilibrium. The samples were filtered with a 0.45 μm membrane filter (Whatman, Dismic-25, Japan) and the absorbance of the filtrate was measured by HPLC.

2.8. Conversion of DTX prodrugs to parent, DTX

The conversion of DTX prodrugs was examined by modification the published paper (Nornoo et al., 2008). Briefly, the conversion reactions were initiated by adding 100 μL of DTX or DTX prodrugs stock solutions (1, 3 and 5 mg/mL) at 0.9 mL of human or rat plasma. The reactions were terminated by adding 0.5 mL of acetonitrile in 0.5 mL of above samples, vortex-mixed for 10 min and centrifuged at 12,000 rpm for 10 min. The upper clear layer was collected and subjected to HPLC analysis.

2.9. Cytotoxicity of DTX or DTX prodrugs as well as vehicles

In vitro anti-proliferation activity was assayed against HepG2 cell lines. Cytotoxicity of DTX or DTX prodrugs was evaluated by MTT method. The experiment was carried out as follows: 100 μL of cell culture medium containing 3×10^3cells was added in each well in a 96-well plate and incubated for 24 h. The confluent wells were treated with DTX or DTX prodrugs (0.1, 1, 5, 10, 50, 100 and 1000 ng/mL).

Simultaneously, the confluent wells were treated with formulations using DTX prodrug (0.01%, 0.1%, 1%, 10%, 25%, 50% and 100%). After 72 h of incubation, the plates were washed with PBS, 100 μL of a MTT (5 mg/mL) solution was added to plates and incubated for a further 2 h at 37 °C. After this, the contents of the plates were dissolved with DMSO and the absorbance was determined at 570 nm in a microplate reader (Sunrise, Tecan, Austria). Cell viability (%) was represented with the (OD of sample-treated cells divided by OD of sample-untreated cells) × 100.

2.10. Determination of DTX-G using various solubilizing agents

The solubility of DTX-G in aqueous or non-aqueous solution was determined by HPLC. Briefly, 1 mg of DTX-G was added to water, water/cosolvent mixtures, water/surfactant mixtures and cyclodextrin solutions, respectively. Various solutions using different cosolvents (ethanol, glycerol, PEG 400 or PG) were prepared at concentrations of 20%, 40%, 60% and 80% (w/w). Various solutions using different surfactants (Tween 80, cremophor EL, poloxamer 188, poloxamer 407 or span 85) were prepared at concentrations of 2.5%, 5%, 10%, 15% and 25% (w/w). Various solutions using different cyclodextrins such as α-CD, β-CD, γ-CD or HPCD were prepared at concentrations of 2.5%, 5%, 10% and 20% (w/w). Then, the samples were stirred for 72 h to reach equilibrium. The resultant suspension was filtered through 0.45 μm-pore sized filter.