Chemistry and Physics of Lipids 188 (2015) 1–9

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

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

# *p*-Aminophenyl-α-D-mannopyranoside engineered lipidic nanoparticles for effective delivery of docetaxel to brain

Indu Singh [a], Rajan Swami [a], Manish Kumar Jeengar [b], Wahid Khan [a,*], Ramakrishna Sistla [a,c,*]

[a] Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad 500037, India
[b] Department of Pharmacology, National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad 500037, India
[c] Medicinal Chemistry and Pharmacology Division, Council of Scientific and Industrial Research-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad 500607, India

ARTICLE INFO

*Article history:*
Received 26 February 2015
Received in revised form 21 March 2015
Accepted 23 March 2015
Available online 25 March 2015

*Keywords:*
Lipid
Blood–brain barrier
*p*-Aminophenyl-α-D-mannopyranoside
Tumor
Solid lipid nanoparticle
Docetaxel
Conjugation

ABSTRACT

Lipidic systems are considered to be the most promising carrier for drug delivery to brain. Metabolic substrates like carbohydrates and amino acids are able to traverse the blood–brain barrier (BBB) by specific carrier-mediated transport systems like glucose transporters present on the both luminal and abluminal side of the BBB. With this objective, the docetaxel (DTX) loaded solid lipidic nanoparticles were formulated and surface modified with a mannose derived ligand *p*-aminophenyl-α-D-mannopyranoside (MAN) to develop MAN conjugated lipidic nanoparticles for targeting DTX to brain. Lipidic nanoparticles were prepared using emulsification and solvent evaporation method using stearic acid as charge modifying lipid and conjugated with MAN using carbodimide coupling. These lipidic nanoparticles were successfully characterized using various techniques like DLS, TEM, DSC and FTIR spectroscopy. Cytotoxicity and cell uptake unveiled enhanced efficacy of conjugated lipidic nanoparticles. Pharmacokinetic and brain distribution studies demonstrated increased DTX concentrations using lipidic nanoparticles in brain and conjugating MAN on surface of lipidic nanoparticles further augmented the inflow of the drug to brain. Present study revealed the prospective of mannose analog, MAN-conjugated lipidic nanoparticles as efficient vehicle for anticancer drug delivery to brain.


## 1. Introduction

Nanotechnology focuses on formulating therapeutic agents in biocompatible nanocomposites such as nanoparticles, nanocapsules, micellar systems and conjugates. Among all the nanoparticulate delivery system, solid lipid nanoparticles (SLN) having the properties of polymeric nanoparticles, fat emulsions and liposomes make SLN a perfect carrier for drug delivery. Lipidic naoparticles are generally made up of biocompatible lipids and natural surfactants (Bondi et al., 2012; Patel et al., 2013; Swami et al., 2013). Surface of these lipidic nanoparticles can be easily modified by the ligands to enhance the targeting efficiency of this delivery system making this system advantageous for active drug targeting (Petros and DeSimone, 2010). Some charge modifying lipids like stearylamine and stearic acid (SA) also provide active groups which act as anchor points for various ligands (Yang et al., 1999; Chen et al., 2001). SLN found renowned attention because of increasing bioavailability potential and targeting to various cancers (Singh et al., 2013). But their nonspecific nature restrict their role as a potential carrier to target cancer cells (Allen and Cullis, 2004). Targeted therapy is an advanced approach for the administration of drugs to specific regions and has received growing attention, not only for the enhancement of therapeutic efficacy but also for the reduction in systemic side effects (Liu et al., 2006). As of now SLN are available in many marketed cosmetic formulations (Pardeike et al., 2009). Solid lipids have been used for several years in the form of pellets in order to achieve a retarded drug release after per oral administration (*e.g.* Mucosolvan® Retard Capsules (Muller et al., 2000). Currently, DSR-MYC, a novel synthetic double-stranded RNA encapsulated stable lipid particle suspension is under phase-II of clinical trials for assessing the safety and efficacy aspects in

* Corresponding authors. Tel.: +91 8790037913 (W. Khan)/+91 4027193753 (R. Sistla).
*E-mail addresses:* wahid@niperhyd.ac.in (W. Khan), sistla@iict.res.in, rksistla@yahoo.com (R. Sistla).

http://dx.doi.org/10.1016/j.chemphyslip.2015.03.003
0009-3084/

solid tumor malignancies (https://clinicaltrials.gov/ct2/show/NCT02110563?term=solid+lipid+particles&rank=1).

Docetaxel (DTX) is a semi-synthetic, taxane derived, highly potent anticancer drug. It has shown broad spectrum antitumor activity against prostate, breast, pancreatic, lung, gastric and hepatic carcinomas (Hwang, 2012; Zhao and Astruc, 2012; Xu et al., 2009a). DTX binds irreversibly with β-actin and stabilizes the microtubule assembly which is responsible for inhibition of cell division and finally cell death (Musumeci et al., 2006). Taxotere®, a marketed formulation of DTX is considered to be potential for brain tumor. However, their side effects may preclude or at least limit their potential clinical application (Schrijvers et al., 1993).

The most important factor limiting the development of new drugs for the central nervous system (CNS) is the blood–brain barrier (BBB). The BBB limits the brain penetration of most CNS drug candidates (Pardridge, 2007). The ability of a particular substance to cross the BBB and enter the brain is dependent upon several factors. But in general, lipophilicity is found to be the key factor for better brain penetration (Bondi et al., 2012). Literature suggests that being lipophilic in nature, lipidic drug carriers easily penetrate the BBB (Oldendorf, 1974).

Most metabolic substrates like sugars and amino acids are able to traverse the BBB by specific carrier-mediated transport systems like glucose transporters (GLUT) present on the both luminal and abluminal side of the BBB (Beduneau et al., 2007; Tsuji, 2005). Overexpression of GLUT1 on glioma cells can give an edge for enhancing the delivery of the cargos to glioma cells (Nishioka et al., 1992; Zeller et al., 1997; Vannucci et al., 1997; Pardridge, 1995). Among all other glucose analogs, mannose can be better recognized by these transporters (Umezawa and Eto, 1988). Understanding the ability of these glucose analogs binding specifically to GLUT1 transporters, give an advantage to use such molecules for active brain targeting in many brain ailments. *p*-Aminophenyl-α-D-mannopyranoside (MAN) is a mannose analog found to have high affinity for GLUT transporters present on BBB. Evaluating the targeting efficiency of MAN, Ying et al. prepared an efficient drug delivery system by conjugating MAN on daunorubicin liposomes for transporting drug across the BBB (Ying et al., 2010). Similarly, Hao et al. also prepared fluorescent dye tagged liposomes modified with MAN to target various functional regions of the brain demonstrating carrier mediated endocytosis potential of MAN (Hao et al., 2013).

Although lipidic carriers can better traverse across the BBB but to improve their targeting efficiency a mannose derivative, MAN which has specific affinity to the GLUT1 transporters, is conjugated to SLN in present work and evaluated for its efficacy through *in vitro* and *in vivo* studies.

## 2. Materials

### 2.1. Reagents and chemicals

Docetaxel (DTX) was a generous gift from Therdose Pharma Pvt., Ltd. (Hyderabad, India). Glyceryl monostearate (GMS) was purchased from Alfa Aesar (Massachusetts, USA). *p*-Aminophenyl-α-D-mannopyranoside, stearic acid (SA), *N*-hydroxysuccinimide (NHS), 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), octadecylamine (ODA), trehalose, cellulose dialysis tubing (molecular weight cut off, 14 kDa) were procured from Sigma–Aldrich (Germany). Soya Lecithin, 30% (SL), fluorescein isothiocyanate isomer I (FITC) were purchased from Himedia (Mumbai, India). Other chemicals were of analytical grade and were purchased from SD-fine Chem Limited (Mumbai, India). U-87 MG cell lines were obtained from National Center for Cell Science (NCCS) (Pune, India). DMEM (Dulbecco's Modified Eagle's Medium), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], trypsin, EDTA were purchased from Sigma Chemicals Co. (St. Louis, MO), Fetal bovine serum were purchased from Gibco, USA, 96 well flat bottom tissue culture plates were purchased from Tarsons Products Pvt., Ltd. (India).

## 3. Methods

### 3.1. Preparation of DTX loaded SLN (U-SLN)

U-SLN were prepared using emulsification and solvent evaporation method (Trotta et al., 2003). Briefly, aqueous phase was prepared by dissolving 150 mg tween 80 in 10 ml of de-ionized water. DTX (10 mg), GMS (85 mg), SA (15 mg) and SL (35 mg) were dissolved in 2 ml of chloroform to get lipid phase in organic solvent and it was added to aqueous phase and was homogenized (Ultra Turrax T25, USA) for 5 min at 12,000 rpm to obtain emulsion. Emulsion, thus obtained, was sonicated (Vibra cell, Sonics, USA) for 15 min to get nanoemulsion. The obtained nanoemulsion was kept under stirring for three hours for evaporation of solvent and finally to get U-SLN.

### 3.2. Conjugation of MAN to U-SLN

MAN was covalently coupled by its amino group to the carboxylic acid group of SA present on the surface of preformed U-SLN. Briefly, 20 mg drug loaded SLN were dispersed in stirring solution of MAN (1 mg) in acidic water (pH 4.75). To this solution EDC and NHS (1:3 molar ratio) were added at room temperature and were allowed to stir for 6 h (Kumar et al., 2001). Excessive unbound MAN and EDC were removed by centrifugation (Sigma Laborentrifugen GMBH, Germany) at 12,000 rpm for 30 min. The nanoparticle pellets were then washed thrice with distilled water, collected and lyophilized using freeze dryer (Skadi, Europe). The lyophilized MAN conjugated SLN were characterized using Fourier transform infrared spectroscopy (FTIR). Conjugation efficiency of MAN–SLN was determined by estimating free or unconjugated MAN content in the supernatant of MAN–SLN dispersion using phenol sulphuric acid method.

### 3.3. FTIR spectroscopy of lipidic formulations

Drug encapsulated lipidic formulations (U-SLN and MAN–SLN) were characterized by means of a FTIR spectrophotometer (PerkinElmer, Spectrum One, USA). The samples were prepared by the potassium bromide disk method and measurements were attempted with the accumulation of 20 scans and a resolution of 4 $cm^{-1}$ over the range of 4000–400 $cm^{-1}$.

### 3.4. Conjugation efficiency

Phenol sulphuric acid method was used to quantify the amount of MAN present on the surface of SLN (Dubois et al., 1956). Briefly, 2 ml aliquot of MAN–SLN supernatant was mixed with 1 ml of 5% aqueous solution of phenol in a test tube. Subsequently, 5 ml of concentrated sulphuric acid was added rapidly to the mixture. After allowing the test tubes to stand for 10 min, they were vortexed for 30 s and placed for 20 min in a water bath at room temperature for color development. Reference solutions were prepared in identical manner as above, except the 2 ml aliquot of supernatant, which was replaced by double distilled water. The phenol used in this procedure was redistilled and 5% phenol in water (w/w) was prepared immediately before the measurement. Absorbances of samples were taken at 487 nm using UV–visible spectrophotometer (Jasco, Japan).