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Delivering etoposide to the brain using catanionic solid lipid nanoparticles with surface 5-HT-moduline

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ABSTRACT

Brain-targeted delivery of etoposide (ETP) is important for treating malignant tumors in the central nervous system. This study presents the transport of ETP across the blood–brain barrier (BBB) using catanionic solid lipid nanoparticles (CASLNs) grafted with 5-HT-moduline. ETP-encapsulated CASLNs (ETP-CASLNs) were prepared in catanionic microemulsion and constructed into solid colloids by rapid cooling. In addition, the uptake of 5-HT-moduline-grafted ETP-CASLNs (5-HT-moduline/ETP-CASLNs) by human brain-microvascular endothelial cells (HBMECs) was visualized by immunochemical staining. We found that a maximal entrapment efficiency of ETP occurred at 0.75 mM of catanionic surfactants. An increase in the concentration of catanionic surfactants reduced the viability of HBMECs. Moreover, an increase in the concentration of 5-HT-moduline reduced the grafting efficiency of 5-HT-moduline, cell viability, and transendothelial electrical resistance of HBMEC monolayer, and enhanced the permeability of propidium iodide and ETP across the BBB. Surface-modified 5-HT-moduline/ETP-CASLNs can be promising drug delivery carriers for anti-brain tumor chemotherapy in preclinical trial.

1. Introduction

An efficacious management of brain cancer is one of inevitable challenges in the current individual healthcare (Kroeger et al., 2010). Several drugs, such as etoposide (ETP), have been applied to chemotherapy for malignant tumors in the central nervous system (CNS). In addition, the antitumor reagents are required to target the blood–brain barrier (BBB) and permeate the neoplasm in brain parenchyma (Pardridge, 2005). Therefore, the transport of pharmaceutical preparations across the BBB is a crucial step in brain tumor treatment (Bredel, 2001). However, the BBB restrains medicinal doses in the circulation system from attaining the curative capacity in the brain (See and Gilbert, 2004). The main traits of the BBB can be attributed to human brain-microvascular endothelial cells (HBMECs), which are responsible for mediating material transfer to shun assaults of toxin on the brain, delivering essential molecules to the CNS, and maintaining the brain homeostasis with normal function of neurotransmitters (Pan and Kastin, 2007). Moreover, the partition characteristics of the BBB results usually from the tight junction on the location between highly impenetrable HBMECs (Kuo and Lu, 2012; Pardridge, 2005). This anatomical structure with aligned adhesive proteins leads to a high transendothelial electrical resistance (TEER), when compared with the cellular configuration in peripheral microvessels (Nag and Begley, 2005; Wolburg and Lippoldt, 2002).

The chemotherapeutic drugs are generally cytotoxic because of their inhibitive ability to cell metabolism and propagation. An encapsulation of pharmaceuticals in carriers can prevent strong adverse reactions. In addition to the reduction in toxic effect on

Abbreviations: 5-HT-moduline, 5-hydroxytryptamine-moduline (tetrapeptide Leu-Ser-Ala-Leu); 5-HT-moduline/ETP-CASLN, 5-HT-moduline-grafted ETP-encapsulated catanionic solid lipid nanoparticle; BBB, blood–brain barrier; CASLN, catanionic solid lipid nanoparticle (with DTMAB and SDS); CB, cacao butter; CNS, central nervous system; DSPE-PEG(2000)-CA, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N- [carboxy(polyethylene glycol)-2000]; DTMAB, decyltrimethylammonium bromide; DYN, dynasan114; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; ELISA, enzyme-linked immunosorbent assay; ETP, etoposide, etopophos, VP-16, 4'-demethylepipodophyllotoxin 9-[4,6-O-(R)-ethylidene-b-L-glucopyranoside]; ETP-CASLN, ETP-encapsulated catanionic solid lipid nanoparticle; HA, human astrocyte; HBME, Chuman brain-microvascular endothelial cell; HPLC, high performance liquid chromatograph; MES, 2-(*N*-morpholino)ethanesulfonic acid; PI, propidium iodide; RMT, receptor-mediated transcytosis; SA, stearic acid; SDS, sodium dodecyl sulfate; SLN, solid lipid nanoparticle; XTT, 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5- carboxanilide.

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Nomenclature

C_S	concentration of catanionic surfactant (mM)
C_{5HTM}	concentration of 5-HT-moduline (μg/mL)
E_{ETP}	entrapment efficiency of ETP in ETP-CASLNs (%)
G_{5HTM}	grafting efficiency of 5-HT-moduline on ETP-CASLNs (%)
P_{CV}	viability of HBMECs incubated with 5-HT-moduline/ETP-CASLNs (%)
P_{DYN}	weight percentage of Dynasan 114 in lipids (%)
P_{BBB}	permeability of ETP across the BBB (cm/s)
P_{PI}	permeability of propidium iodide across the BBB after incubation with 5-HT-moduline/ETP-CASLNs (cm/s)
TEER	transendothelial electrical resistance ($\Omega \times cm^2$)
ζ	zeta potential of 5-HT-moduline/ETP-CASLNs (mV)

normal tissue, the formulated carriers can enhance the drug delivery to targeted cells. The application of colloidal system containing lipids of high melting point can carry hydrophobic agents, reduce toxicity, and produce biomimetic solid vehicles (Jones and Shusta, 2007; Mehnert and Mader, 2001). Solid lipid micropellets was first developed about two decades ago and modified into solid lipid nanoparticles (SLNs) reserving the quality of polymer and liposome nanocarriers for drug administration (Eldem et al., 1991; Kuo and Su, 2007; Muller et al., 1997). It has been observed that SLNs reduced the elimination rate and promoted the release efficiency of anticancer doxorubicin to the brain of rodents (Fundaro et al., 2000; Wang et al., 2002). It has been also found that SLNs prolonged the residence period of cytotoxic camptothecin in the brain (Yang et al., 1999). Moreover, the charge regulation on the self-assembled surface of catanionic SLNs (CASLNs), comprising conjugated cationic and anionic surfactants, could improve the delivery efficiency of carmustine and doxorubicin to glioblastoma multiforme (Kuo and Liang, 2011a; Kuo and Liang, 2011b). CASLNs were also demonstrated as an efficient drug delivery system for transporting saquinavir across the BBB (Kuo and Wang, 2014).

The biophysical and biochemical properties of drug carriers can strongly affect the receptor-mediated transcytosis (RMT), an important pathway for regulating the drug delivery to HBMECs (Abbott, 2004; Abbott, 2005; Gabathuler, 2010; Kuo and Lee, 2012; Kuo and Yu, 2011b). In fact, the use of specific ligand-binding receptor is promising in brain-targeted delivery. The 5-HT_{1B} receptor, expressed by brain endothelia, plays an essential role in physiological evolution (Lin and Parsons, 2002). For example, 5-HT_{1B} receptor activation could enhance the mobility of mice, suggesting a potential approach to the treatment of depression (Svenningsson et al., 2006; Tatarczynska et al., 2005). The 5-HT_{1B} receptor could also affect neural and vascular inflammatory responses in migraine. It has been observed that 5-HT moduline, an allosteric tetrapeptide modulator, could modify the function of 5-HT_{1B} receptor on inhibiting the binding of 5-HT and generate an antagonist-like effect on the 5-HT_{1B} receptor (Massot et al., 1996; Rouselle et al., 1996). Thus, the interaction between 5-HT moduline and 5-HT_{1B} receptor recommended a therapeutic strategy for delivering drugs via the RMT pathway.

The aim of this study was to demonstrate the ability of CASLNs with surface 5-HT moduline to improve the targeting delivery of ETP across the BBB. The release of ETP to the brain is an important topic in the antitumor treatment. We investigated the particle size distribution, zeta potential, grafting efficiency of 5-HT moduline, entrapment efficiency of ETP, cytotoxicity to HBMECs, particle impact on the tight junction, uptake of fluorescent carriers, and permeability of ETP across the BBB.

2. Materials and methods

2.1. Preparation of ETP-CASLNs

Decyltrimethylammonium bromide (DTMAB; Fluka, Buchs, Switzerland) was mixed with sodium dodecylsulfate (SDS; Sigma, St. Louis, MO) in equimolar scheme at 0.25, 0.75, 1, 1.25, 1.5, 1.75, and 2 mM in ultrapure water (Barnstead, Dubuque, IA). The lipid phase was prepared by dissolving Dynasan 114 (DYN; Sigma), cacao butter (CB; OCG Cacao, Whitinsville, MA), stearic acid (SA; octadecanoic acid; hexyldecyl stearate; Sigma), and ETP (5% (w/w), Sigma) in methanol (J. T. Baker, Phillipsburg, NJ) at 75 °C and 400 rpm. The weight percentage of DYN in lipids was controlled at 0%, 33%, 67%, and 100%. In addition, the same weight percentage of CB and SA in lipids was used. The aqueous phase of 750 μL containing catanionic surfactants and the lipid phase of 250 μL containing 1.25% (w/w) 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[carboxy (polyethylene glycol)-2000] (DSPE-PEG(2000)-CA; Avanti Polar Lipid, Alabaster, AL) and 4.75% (w/w) 1-butanol (Riedel-de Haen, Seelze, Germany) were emulsified at 75 °C and 400 rpm for 30 min. Fluorescein isothiocyante-conjugated dextran 70,000 (0.1% (w/v), Sigma) was added into the aqueous phase for producing fluorescent ETP-loaded CASLNs (ETP-CASLNs). The microemulsified fluid of 1 mL was added gradually into ultrapure water of 10 mL at 3 °C and 400 rpm for 15 min.

2.2. Entrapment efficiency of ETP in ETP-CASLNs

The suspension containing ETP-CASLNs was filtrated through a filter with pores of 2 μm and separated using a centrifuge (AVANTij-25, Beckman Coulter, Palo Alto, CA) at 159,000 × *g* and 25 °C for 10 min. The pellet was resuspended in ultrapure water containing 1% (w/v) L-mannitol (Sigma), refrigerated in a freezer (Sanyo, Osaka, Japan) at −80 °C for 30 min, and lyophilized using a freeze dryer (Eyela, Tokyo, Japan) at 2–4 torr and −80 °C for 24 h. The quantity of free ETP in the supernatant was determined using a high performance liquid chromatograph (HPLC; Jasco, Tokyo, Japan) connected to an ultraviolet (UV) detector (Jasco) at 284 nm. A reverse phase column (BDS Hypersil C-18, Thermo Hypersil-Keystone, Bellefonte, PA) of the HPLC was sheathed in a column heater (Alltech, Derrfield, IL) at 45 °C. The mobile phase in the column contained acetonitrile (BDH, Poole, England) gradient from 5% to 50% (v/v) and was propelled using two high pressure pumps (PU-2080 Plus, Jasco) in series at a flow rate of 0.85 mL/min for 20 min. The entrapment efficiency of ETP in ETP-CASLNs, E_{ETP}, is defined as E_{ETP} (%) = $[(W_{t,ETP} - W_{s,ETP})/W_{t,ETP}] \times 100\%$, where $W_{t,ETP}$ and $W_{s,ETP}$ are the total weight of ETP in each batch and the weight of supernatant ETP in each batch, respectively.

2.3. Preparation of 5-HT-moduline/ETP-CASLNs

ETP-CASLNs were suspended in 2-(*N*-morpholino) ethanesulfonic acid (MES; Sigma) and activated by mixing with *N*-hydroxysulfosuccinimide (Thermo Fisher Scientific, Rockford, IL) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC; Sigma) in a molar ratio of DSPE-PEG(2000)-CA to EDC being 1:5 at 25 °C and 80 rpm for 1 h. The suspension was stored at 4 °C for 24 h and centrifuged at 159,000 × *g* and 4 °C for 30 min. The bottom pellet was resuspended in MES and mixed with 5-HT-moduline (Kelowna International Scientific, Taipei, Taiwan) at 4 °C and 80 rpm for 6 h, dialyzed using a tube of 100 kDa, frozen, and lyophilized. The concentration of 5-HT-moduline was 10, 20, and 30 μg/mL. The quantity of free 5-HT-moduline was determined with a