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Using catanionic solid lipid nanoparticles with wheat germ agglutinin and lactoferrin for targeted delivery of etoposide to glioblastoma multiforme

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ABSTRACT

Catanionic solid lipid nanoparticles (CASLNs) with surface wheat germ agglutinin (WGA) and lactoferrin (Lf) were formulated to entrap and release etoposide (ETP), cross the blood–brain barrier (BBB), and inhibit glioblastoma multiforme (GBM) growth. Microemulsified ETP-CASLNs were modified with WGA and Lf to permeate a cultured monolayer of human brain-microvascular endothelial cells (HBMECs) regulated by human astrocytes and to treat malignant U87MG cells. Experimental evidence revealed that an increase in the weight percentage of ETP from 1% to 4% decreased its encapsulation efficiency about 34-44%. In addition, the release rate of ETP from WGA-Lf-ETP-CASLNs decreased with an increase in the concentration of catanionic surfactant from 7.5 μM to 12.5 μM, and WGA-Lf-ETP-CASLNs at 12.5 μM of catanionic surfactant exhibited a feature of sustained release. WGA-Lf-ETP-CASLNs also reduced transendothelial electrical resistance from 245.5 $\Omega\times cm^2$ to 191.5 $\Omega\times cm^2$, enhanced the permeability of propidium iodide from 3.62×10^{-6} cm/s to 5.61×10^{-6} cm/s, induced a minor cytotoxicity to HBMECs, increased the ability of ETP to cross the BBB by about 5.6 times, and improved the antiproliferative efficacy of U87MG cells. The grafting of WGA and Lf is crucial to control the medicinal property of ETP-CASLNs, and WGA-Lf-ETP-CASLNs can be promising colloidal carriers in GBM management.

Abbreviations

BBB	blood–brain barrier
BMEC	brain-microvascular endothelial cell
BW	beeswax
CASLN	catanionic solid lipid nanoparticle
CNS	central nervous system
ETP	ETP
ETP-CASLN	ETP-loaded catanionic solid lipid nanoparticle
HA	human astrocyte
HBMEC	human brain-microvascular endothelial cell
HBMEC/HA	HBMECs regulated by HAs
Lf	lactoferrin
Lf-ETP-CASLN	ETP-loaded catanionic solid lipid nanoparticle with surface lactoferrin
LfR	lactoferrin receptor
PI	propidium iodide
RME	receptor-mediated endocytosis
TEER	transendothelial electrical resistance
WGA	wheat germ agglutinin
WGA-ETP-CASLN	ETP-loaded catanionic solid lipid nanoparticle with surface wheat germ agglutinin
WGA-Lf-ETP-CASLN	ETP-loaded catanionic solid lipid nanoparticle with surface wheat germ agglutinin and lactoferrin

1. Introduction

Glioma is classified as one of the frequently encountered causes of intracranial carcinoma [1]. Of the four grades of glioma, glioblastoma multiforme (GBM) is the most malignant at grade IV, with patients living an average of 1–1.5 years after diagnosis [2,3]. The major problems in treating GBM are the difficulty of early-stage diagnosis, the location of the GBM next to tissue regulating basic neurophysiology, the aggressiveness of the phenotype in the central nervous system (CNS), angiogenetic propagation, rapid progression, unsatisfactory prognosis, and a high probability of recurrence [4–6]. Therefore, effective GBM management is a crucial challenge to clinical practice in brain pathology. In addition, conventional chemotherapy for GBM can be inefficacious because the ability of antimitotic drugs, such as etoposide (ETP), to cross the blood–brain barrier (BBB) is low [7]. Hence, a promoted BBB permeability is essential to inhibit GBM growth in the CNS. The use of targeting molecules can be a practical response to this

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Nomenclature

C_{Lf}	concentration of Lf for grafting on ETP-CASLNs (μg/mL)
C_s	concentration of catanionic surfactant (μM)
C_{WGA}	concentration of WGA for grafting on Lf-ETP-CASLNs (mg/mL)
D	average diameter of WGA-Lf-ETP-CASLNs (nm)
D_{ETP}	cumulative percentage of ETP dissolved from WGA-Lf-ETP-CASLNs (%)
E_{ETP}	encapsulation efficiency of ETP in CASLNs (%)
E_{Lf}	grafting efficiency of Lf on ETP-CASLNs (%)
E_{WGA}	grafting efficiency of WGA on Lf-ETP-CASLNs (%)
P_{BW}	weight percentage of beeswax in ETP-CASLNs (%)
E_{WGA}	grafting efficiency of WGA on Lf-ETP-CASLNs (%)
$P_{ETP,BBB}$	ability of ETP to cross the BBB after treatment with WGA-Lf-ETP-CASLNs (cm/s)
P_{ETP}	weight percentage of ETP in ETP-CASLNs (%)
$P_{PI,BBB}$	ability of PI to cross the BBB after treatment with WGA-Lf-ETP-CASLNs (cm/s)
$P_{V,HBMEC}$	viability of HBMECs after treatment with WGA-Lf-ETP-CASLNs (%)
$P_{V,U87MG}$	viability of U87MG cells after treatment with WGA-Lf-ETP-CASLNs (%)
t	time consumed in releasing ETP from WGA-Lf-ETP-CASLNs (h)
ζ	zeta potential of WGA-Lf-ETP-CASLNs (mV)

problem. For example, wheat germ agglutinin (WGA) on poly(ethylene glycol)-poly (lactic acid) nanoparticles could be effective in delivering a drug across the BBB through its immobilized carbohydrate-binding pockets [8]. It is worthy of note that the substances in wheat can be important natural bioresources in medicine and agriculture [9–14]. WGA was also capable of interacting with an *in vitro* BBB model composed of human epithelial ECV304 [15]. Moreover, lactoferrin receptor (LfR) on brain-microvascular endothelial cells (BMECs) could facilitate the infiltration of lactoferrin (Lf) across the BBB via receptor-mediated endocytosis (RME) [16]. In addition to BMECs, it was reported that GBM cells also expressed LfR [17].

Catanionic solid lipid nanoparticles (CASLNs) can be stable particles because they have a unique microstructure with a composite interface of an amphoteric charge. In colloidal science, CASLNs evolved from emulsion of vesicles or micelles that contained zwitterionic surfactant [18]. Cancellation of the opposite charge of polar groups and conjugation of hydrocarbon chains could decrease the energy barrier and lead to a high affinity between catanionic micelles in an isometric cluster formation [19]. In addition, the physicochemical behavior of catanionic surfactant could be explained by considering three major components (oil, water, and alcohol) of microemulsion [20]. In fact, a transition enthalpy study indicated that catanionic surfactants could be spontaneously self-assembled into aggregates [21]. Smoluchowski's theory of Brownian flocculation also revealed that in a non-equilibrium state, disk-like bilayer fusion could be faster than catanionic vesicle coalescence [22]. Moreover, an emulsified system of catanionic surfactant could produce wormlike micelles [23]. In pharmaceutical formulation, these complicated colloids are important carriers for drug loading and dissolution [24]. Catanionic aggregates, for instance, were regarded as an effective dosage form for prolonged release [25]. Furthermore, a cytotoxicity study reported that catanionic aggregates could injure cell membrane during fusional internalization and induce the apoptotic pathway of 3T6 and HeLa cells [26]. Positively charged catanionic vesicles were also able to vary the cell size of human mononuclear U-937 macrophages and their mitochondrial membrane potential [27]. It was found that CASLNs carrying carmustine could significantly reduce the malignancy of GBM [28]. Thus, CASLNs can be qualified carriers in encapsulating hydrophobic ETP and in controlling its delivery.

The aim of this study was to develop ETP-loaded CASLNs (ETP-CASLNs) for sustained release of ETP, a chemotherapeutic agent, and to develop WGA- and Lf-grafted ETP-CASLNs (WGA-Lf-ETP-CASLNs) for targeting delivery to CNS tumors. Since catanionic surfactant can mediate charge behavior on the particle surface, and WGA and Lf can be functional in brain-targeting transport, it would be important to investigate WGA-Lf-ETP-CASLNs as a colloidal delivery system for GBM therapy. In addition, anticancer drugs are generally lipophilic with poor stability. They are usually eliminated in the blood and generate side responses in the human body, and their brain uptake is limited by the BBB [29,30]. In this study, the potential of CASLNs, acting as ETP carriers, to prolong the efficacy of ETP in the circulation system and reduce cytotoxicity was investigated. CASLNs grafted with WGA and Lf may have a dual ability to cross the BBB and conjugate tumor cells for better GBM management. We examined the particle size distribution, surface charge, particle structure, and encapsulation efficiency of ETP, the dissolution kinetics of ETP, the integrity of the BBB after treatment with WGA-Lf-ETP-CASLNs, the ability of ETP to cross the BBB, toxicity to human BMECs (HBMECs), and efficacy against propagation of U87MG cells.

2. Materials and methods

2.1. Materials

Beeswax (BW), behenic acid (BA; docosanoic acid), D-mannitol, Dulbecco's phosphate-buffered saline (DPBS), ETP, ethylenediaminetetraacetic acid (EDTA), phosphotungstic acid (PTA), sodium dodecylsulfate (SDS), sodium azide, stearic acid (SA; octadecanoic acid), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), bicinchoninic acid (BCA) assay kit, propidium iodide (PI), and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma-Aldrich (St. Louis, MO). Ethanol was obtained from Tedia (Fairfield, OH). 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (DSPE-PEG(2000)-CA) was purchased from Avanti Polar Lipid (Alabaster, AL). *N*,*N*-di-(b-stearoylethyl)-N,N-dimethyl-ammonium chloride (esterquat 1; EQ 1) was obtained from Gerbu Biotechnik (Gaiberg, Germany). *N*-hydroxysuccinimide (NHS) and Triton-X-100 were purchased from Acros (Morris, NJ). Tris hydroxymethyl aminomethane (Tris) was purchased from Riedel-de Haen (Seelze, Germany). HBMECs were obtained from Biocompare (South San Francisco, CA). Human astrocytes (HAs) were purchased from Sciencell (Corte Del Cedro Carlsbad, CA). U87MG cells were obtained from Bioresource Collection and Research Center (Hsin-Chu, Taiwan). 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) was purchased from Biological Industries (Beit Haemek, Israel). Transwell and polyethylene terephthalate (PET) membrane were obtained from BD Falcon (Franklin Lakes, NJ). Anti-LfR monoclonal antibody, anti-*O*-linked *N*-acetylglucosamine antibody, and goat polyclonal secondary antibody to mouse immunoglobulin G (heavy and light) (IgG (H+L)) with rhodamine were obtained from Abcam (Cambridge, MA).

2.2. Preparation of WGA-Lf-ETP-CASLNs

BW, BA, SA, DSPE-PEG(2000)-CA and ETP were homogenized at 150 rpm and 75 °C for 10 min. The weight percentage of BW was 25%, 50%, or 75% in the lipid phase, and that of ETP was 1%, 2%, or 4%. The weight percentage of DSPE-PEG(2000)-CA was 1.25% in the

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