Colloids and Surfaces B: Biointerfaces 161 (2018) 497–507

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Full Length Article

Characterization of liposome-containing SPIONs conjugated with anti-CD20 developed as a novel theranostic agent for central nervous system lymphoma

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ARTICLE INFO

Article history:
Received 12 June 2017
Received in revised form 19 October 2017
Accepted 1 November 2017
Available online 8 November 2017

Keywords:
Primary central nervous system lymphoma (PCNSL)
Blood brain barrier (BBB)
Brain lymphoma
Superparamagnetic iron oxide nanoparticle (SPIONs)
Rituximab
Anti-CD20
Liposome
Theranostics

ABSTRACT

Despite advances in neuroscience cancer research during the past decades, the survival of cancer patients has only marginally improved and the cure remains unlikely. The blood-brain barrier (BBB) is a major obstacle protecting the entry of therapeutic agents to central nervous system, especially for primary central nervous system lymphoma (PCNSL). Thus, the use of small nanoparticle as a drug carrier may be new strategies to overcome this problem. In this study, we fabricated liposome consisting of superparamagnetic iron oxide nanoparticles (SPIONs) functionalized with anti-CD20 (Rituximab; RTX). The designed nanoparticles have a theranostic property which is not only to improve drug delivery, but also to offer diagnostic and monitoring capabilities. TEM images revealed the spherical shape of liposome with the approximately average diameters about 140–190 nm with slightly negatively charge surfaces. Superparamagnetic property of SPIONs-loaded liposomes was confirmed by VSM. Liposome colloidal could be prolonged at 4 °C and 25 °C storages. RTX conjugated liposome induced cell internalization and apoptosis effect in B-lymphoma cells. Drug targeting and therapeutic effect was investigated in BBB model. The result confirmed that liposome nanocarrier is required as a drug carrier for effectively RTX across the BBB.

1. Introduction

Despite advances in the understanding of genetic and molecular abnormalities relating to cancer, and in development of new therapies, the prognosis of patients with primary central nervous system lymphoma (PCNSL) remains poor when compared to other lymphoma types (median survival 3–5 years) [1]. For immunocompromised patients, such as those with AIDS or in cases of organ transplantation, median survival times are often markedly reduced (typically less than 1 year) [2]. Due to this, and the rising incidence of PCNSL over the past decades there is a need to develop new diagnostic and therapeutic strategies which are applicable to these patients, including the wider population [3].

PCNSL accounts for 3–5% of primary brain tumors in adults, and occurs with an incidence of 5 per 1,000.000 people in developed countries [1]. Most (>95%) PCNSL cases are of the diffuse large B-cell type. Genetic abnormalities in PCNSL cells are distinct from those occurring in other lymphomas; this factor may relate to observed differences in prognosis and treatment response [4]. Non-invasive imaging studies such as magnetic resonance imaging (MRI) and positron-emission tomography (PET) scans can support an initial diagnosis of PCNSL, however confirmation usually requires a stereotactic biopsy. Alternatively, cytology of cerebrospinal fluid, or ocular vitreous, may confirm diagnosis in 20–30% of patients. Diagnosis of AIDS-associated PCNSL represents an important challenge in clinical practice. Radiographic findings of PCNSL in immunocompromised patients are often indistinguish-

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https://doi.org/10.1016/j.colsurfb.2017.11.003
0927-7765/

able from infections, particularly toxoplasmosis. In these cases, biopsies are often deferred until the empiric treatment for toxoplasmosis fails, causing delays in diagnosis. Therefore, non-invasive diagnostic approaches, such as neuroimaging probes, for PCNSL in AIDS patients may hasten diagnosis allowing early treatment. High-dose methotrexate (HD-MTX)-based chemotherapy regimens are the standard treatment for PCNSL [5], and these combined with other chemotherapies (cytarabine, procarbazine, vincristine, or ifosfamide) have been shown to provide superior results in some cases, although the higher toxicity is a serious issue. Rituximab (RTX), a monoclonal antibody against CD20, has demonstrated promising activity as a single agent in PCNSL [6], despite the inherent limitation of such a large molecule in traversing the blood-brain barrier [7,8]. Employing nanoparticles as a carrier for RTX may help overcome this challenge, and result in increased efficacy in PCNSL treatment.

The blood-brain barrier represents a major obstacle for drug delivery to the CNS [9,10]. The use of a small lipophilic carrier, such as a nanoparticle system, may overcome this problem, with nanoparticles allowing for improved drug delivery metrics as well as diagnostic and monitoring capabilities. This emerging molecular platform, "theranostics" [11], employs nanoparticle systems based on liposomes [12], micelles [13], and dendrimers [14] to protect drugs and deliver them to targets in a controlled manner. In addition, they can be decorated with "molecular antennae" such as antibodies or aptamers on their surface to allow target specificity. Imaging probes are an important group of theranostic particles: these can be detected in vivo through various imaging modalities [15]. Superparamagnetic iron oxide nanoparticles (SPIONs) and radioisotopically labeled nanoparticles are common imaging probes detectable by magnetic resonance imaging and positron emission tomography, respectively. Among the various nanoparticle classes available, liposomes have shown real promise in the treatment of brain disorders [16–18], having been shown to enhance the delivery of neuroprotective agents to the *peri*-infarct area in experimental ischemic stroke models [17,18]. Surface-modified liposomes have been developed to increase drug delivery into the brain. So far, surface conjugation of liposome with transferrin is a common BBB delivery system by exploiting transferrin receptors at the blood-brain barrier to mediate transcytosis of liposomes. [19]. Liposome conjugated with transferrin antibody containing P53 plasmid (SGT-53, SynerGene Therapeutics, USA) has been advanced into phase II clinical trial evaluation in patients with recurrent glioblastoma. In addition, transferrin-neuroprotective agents [20–22] have been tested in preclinical models of Alzheimer's disease.

Other surface modification platforms to improve BBB penetration of liposomes include lactoferrin [23], glucose [24] and glutathione [25]. At present, diagnosis and treatment of CNS lymphoma remains a significant challenge and represents an unmet clinical need. Diagnosis relies on brain biopsy. Non-invasive imaging technique that can aid diagnosis is urgently needed particularly for immunocompromised patients e.g. AIDS, whom clinical and conventional radiographic findings of CNS lymphoma are indistinguishable from other mimics such as toxoplasmosis. In addition, novel treatment strategies to improve survival outcome with less toxicity are critically required for patients with advanced age or poor functional status. In this study, we have successfully designed and fabricated a novel theranostic nanoparticle system consisting of liposome containing SPION (for tracking and treatment monitoring) functionalized with RTX (for specific targeting and treatment) and coated with a surfactant, tween 80 (to improve BBB penetration). In addition, our nanoparticle system has potential to exhibit anti-cancer immune response [26] and serve as a substrate (SPIONs) for thermal ablation.

2. Materials and methods

2.1. Materials

Soybean lecithin (Soya Phosphatidylcholin; PC) was purchased from Degussa (Hamburg, Germany). 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polythylene glycol 2000)] (DSPEG-PE), and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[4-(*p*-maleimidophenyl)butyramide] (MPB-PE, linker) were purchased from Avanti Polar Lipids, Inc (Alabama, USA). Lipid molecular structures used in this study were showed in Supplementary materials. Cholesterol was also obtained from Avanti Polar Lipids, Inc (Alabama, USA). Polysorbate 80 (Tween 80) was obtained from Sigma-Aldrich (Saint Louis, MO, USA). Phosphate buffered saline (pH 7.4) (PBS; containing 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4; 2 mM KH_2PO_4), and Triton X-100 were obtained from Merck (Merck Millipore, Darmstadt, Germany). Anti-CD20 (RTX) was purchased from Roche (Basel, Switzerland). Roswell Park Memorial Institute media (RPMI 1640) was from GIBCO Invitrogen (New York, USA). Fetal bovine serum (FBS) was obtained from Biochrom AG (Berlin, Germany). Trypsin-EDTA, L-glutamine, penicillin G sodium, streptomicin sulfate, and amphotericin B were obtained from Invitrogen Corp. (New York, USA). MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was purchased from GIBCO Invitrogen (NY, USA). Dimethylsulfoxide (DMSO) was procured from Sigma-Aldrich, Inc, (Dorset, UK). The Annexin V-FITC Apoptosis Detection Kit was obtained from NeXins Research BV (Rotterdam, the Netherlands). Distilled water was generated using an ELGA system (PureLab Ultra, Illinois, USA). Z138C and Granta519 lymphoma cell lines were graciously provided by Dr. Siwanon Jirawatnotai (Bangkok, Thailand). SPIONs-PVA nanoparticles were synthesized from maghemite (γ-Fe_2O_3), affording SPIONs having a diameter less than 20 nm. All SPIONs were coated with PVA (Polyvinyl alcohol)-OH for stabilization [27–29] and called SPION-PVA.

2.2. Lymphoma cell cultivation

Granta and Z138C cell lines were cultured in RPMI 1640 medium. The medium was supplemented with 10% FBS containing 0.1 mM non-essential amino acids (100 μg/ml L-glutamine, 100 μg/ml streptomycin and 100 U/ml penicillin). Cells were grown and propagated in 75 ml T-flasks, and incubated at 37 °C in a humidified atmosphere containing 5% CO_2. The medium was changed every other day.

2.3. Preparation of liposomes and conjugation of RTX

A series of liposome nanoparticles were prepared by conventional thin film hydration, which involved the mixing of PC, DSPEG-PE, Tween 80 and MPB-PE (linker) in various proportions, as indicated in Table 1. Each mixture was then dissolved in chloroform-diethyl ether (3:1 v/v, 10 ml), and upon solvent removal by rotary evaporation at 25 °C under 50–100 kg/cm^2 nitrogen flow thin lipid films were obtained. After drying, lipid films were rehydrated with 0.2 mg_{Fe}/ml of SPION-PVA dissolved in PBS (pH 7.4), and re-suspended with shaking at room temperature. Particle sizes of all liposome samples were reduced using a discontinuous extruder (Liposo-Fast^{TM}-10, Avestin Inc, Ottawa, Canada) operating at 200 bar pressure over 15–20 cycles, through a 200 nm pore size polypropylene membrane (Millipore GmbH, Eschborn, Germany). The SPIONs-PVA loaded liposomes were purified by centrifugation (TomyMX-301, Tokyo, Japan) at 9840 g for 30 min, prior to collection. The obtained pellets were re-suspended in PBS (pH 7.4) for measuring of SPION entrapment. Additionally, in order to obtain an optimized formulation for BBB delivery, the