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Solid lipid nanoparticles with surface antibody for targeting the brain and inhibiting lymphatic phagocytosis

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ABSTRACT

Solid lipid nanoparticles (SLNs) with surface monoclonal antibody (MAb), poloxamer 407 (P407), and polysorbate 80 (Tween 80) (defined as P-T-MAb/SQV-SLNs) were used to enhance the transport efficacy of antiviral saquinavir (SQV) across the blood–brain barrier (BBB). Internalizations of P-T-MAb/SQV-SLNs by RAW264.7 cells and by human brain-microvascular endothelial cells were quantified by chemical assay and examined by immunochemical staining. The results revealed that a decrease in the weight percentage of Dynasan 114 (DYN) in internal lipids, comprising DYN, palmitic acid, and cacao butter, increased the particle size and zeta potential of P-T-MAb/SQV-SLNs. In addition, the external P407 and Tween 80 could stabilize P-T-MAb/SQV-SLNs and reduce phagocytosis by RAW264.7 cells. Tween 80 on P-T-MAb/SQV-SLNs also benefited the delivery of SQV across the BBB. Moreover, MAb grafted on P-T-MAb/SQV-SLNs promoted the permeability of SQV across the blood–brain barrier. The grafting of MAb and coating of P407 and Tween 80 on the surface of SLNs demonstrate an effective strategy to prevent phagocytosis by RAW264.7 cells and to promote the targeting delivery of SQV across the BBB for inhibiting retroviral growth.

1. Introduction

The therapy for acquired immunodeficiency syndrome (AIDS) is a global challenge to biomedicine and public hygiene [1]. The pathogen of AIDS is human immunodeficiency virus (HIV), a chronic retrovirus with size of 100–150 nm [2]. HIV, comprising lipid membrane, transcriptase, nucleocapsid, and ribonucleic acid (RNA), resides in the brain parenchyma of AIDS patients [3]. [[[N-(2-quinoly-carbonyl)-L-asparaginyl]amino]butyl]-(4aS,8aS)-iso-quinoline-3(S)-carboxamide methane-sulfonate (saquinavir, SQV) is a protease inhibitor for treating AIDS via binding polypepton expressed by gag and gag-pol genes of viral RNA [4]. However, the permeability of SQV across the blood–brain barrier (BBB) is extremely low, rendering a restricted therapeutic efficacy in clinical practice [5–8].

Solid lipid nanoparticles (SLNs) belong to submicron drug delivery systems with diameter ranging from 100 nm to 1000 nm and can be prepared by microemulsified system and high pressure homogenization [9]. The surfactants used in developing SLNs were generally regarded as safe components in pharmaceutical formulation without perceptible toxicity [10]. In addition, SLNs can effectively grasp hydrophobic remedies in their internal phase and retain the advantages of colloidal carriers, including physical stability, medicine-protective effect, controlled release, good biocompatibility, specific delivery, and scale-up feasibility [11,12]. However, a rapid elimination by mononuclear phagocytes and a probable low endocytotic uptake by diseased nidus tissue were the main drawbacks of SLNs [13]. When phagocytes internalize SLNs, reticuloendothelial system (RES) organs often

Abbreviations: AIDS, acquired immunodeficiency syndrome; BBB, blood–brain barrier; BCA, bicinchoninic acid; CB, cacao butter; DSPE-PEG(2000)-CA, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000]; DYN, Dynasan 114; ELISA, enzyme-linked immunosorbent assay; FE-SEM, field emission scanning electron microscope; HA, human astrocyte; HBMEC, human brain-microvascular endothelial cell; HIV, human immunodeficiency virus; HPLC, high performance liquid chromatograph; MAb, monoclonal antibody; MAb/SQV-SLNs, monoclonal antibody-grafted solid lipid nanoparticle with saquinavir; P-T/SQV-SLN, poloxamer 407- and polysorbate 80-stabilized solid lipid nanoparticle with saquinavir; P-T-MAb/SQV-SLN, poloxamer 407- and polysorbate 80-stabilized monoclonal antibody-grafted solid lipid nanoparticle with saquinavir; P407, poloxamer 407; PA, palmitic acid; PSG, penicillin–streptomycin–glutamine; RES, reticuloendothelial system; SLN, solid lipid nanoparticle; SQV, saquinavir, [[[N-(2-quinoly-carbonyl)-L-asparaginyl]amino] butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxamide methane-sulfonate; SQV-SLN, saquinavir-loaded solid lipid nanoparticle; XTT, 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide.

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accumulate the internalized SLNs, depriving their further circulation possibility [14]. To overcome this tough problem, a modification of surface characteristics can be a solution via altering the fate of SLNs after intravenous injection. It has been observed that poloxamers (such as poloxamer 407 (P407)) and polysorbates (such as polysorbate 80 (Tween 80)) could prevent the phagocytotic uptake of polymethyl methacrylate nanoparticles [15]. Moreover, it has been concluded that monoclonal antibody (MAb) could potentially target insulin receptors on human brain-microvascular endothelial cells (HBMECs) and improve the brain-targeting efficiency [16].

The aim of this work is to verify ameliorated receptor-mediated transcytosis into HBMECs and reduced lymphatic phagocytosis by grafting MAb, P407, and Tween 80 on SQV-loaded SLNs (P-T-MAb/SQV-SLNs). To hinder HIV propagation in the brain, the elimination of drug-carrying SLNs by RES and the low bioavailability of SQV are two thorny problems. These issues are resolved using P-T-MAb/SQV-SLNs in this study. We investigated the particle size distribution, zeta potential, surface morphology, entrapment efficiency of SQV, grafting efficiency of MAb, phagocytosis, and endocytosis of P-T-MAb/SQV-SLNs. In addition, the uptake percentage of P-T-MAb/SQV-SLNs by RAW264.7 cells and the permeability of SQV across the BBB were evaluated.

2. Materials and methods

2.1. Fabrication of P-T-MAb/SQV-SLNs

4% (w/v) lipids, including Dynasan 114 (DYN; Sigma, St. Louis, MO), palmitic acid (PA; Sigma), and cacao butter (CB; OCG Cacao, Whitinsville, MA), were mixed with 1.25% (w/v) 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (DSPE-PEG(2000)-CA; Avanti Polar Lipid, Alabaster, AL), and 4% (w/v) SQV (United States Pharmacopeial, Rockville, MD) in methanol (J. T. Baker, Phillipsburg, NJ) at 400 rpm and 75 °C. In the case of equal weight of PA and CB, the weight percentage of DYN in the lipid phase was controlled at 0%, 33%, 67%, and 100%. 0.4% (w/v) cholesteryl hemisuccinate (Sigma), 0.2% (w/v) taurocholate (Sigma), 0.4% (w/v) L-A-phospatidylcholine type II-S (Sigma), 0.5% (w/v) P407 (Sigma), 0.5% (w/v) Tween 80 (Sigma), and 2% (v/v) n-butanol (Riedel-de Haen, Seelze, Germany) were mixed in ultrapure water (Barnstead, Dubuque, IA) at 400 rpm and 75 °C. P407- and Tween 80-free SQV-SLNs were obtained without adding the two surfactant components in the aqueous phase. 0.1% (w/v) fluorescein isothiocyanate-conjugated dextran 70000 (Sigma) was added into the aqueous phase for preparing fluorescent P407- and Tween 80-stabilized SLNs with SQV (P-T/SQV-SLNs). 250 μL of the lipid phase was added slowly into 1250 μL of the aqueous phase at 400 rpm and 75 °C for 10 min. The micro-emulsified liquid was added into 15 mL of ultrapure water at 1000 rpm and 3 °C for 15 min to solidify particles and filtrated through a filtration paper with pores of 1 μm. The filtrate was centrifuged by a superspeed refrigerated centrifuge (AVANTij-25, Beckman Coulter, Palo Alto, CA) at 159,000 × *g* and 4 °C for 10 min. The pellet was suspended in ultrapure water with 2% (w/v) D-mannitol (Sigma), frozen in an ultralow temperature freezer (Sanyo, Osaka, Japan) at −80 °C for 1 h, and lyophilized by a freeze dryer (Eyela, Tokyo, Japan) at 2–4 Torr and −80 °C for 24 h. The supernatant was used to estimate the entrapment efficiency of SQV via separating by a high performance liquid chromatograph (HPLC; Jasco, Tokyo, Japan) with a reverse phase BDS Hypersil C-18 column (Thermo Hypersil-Keystone, Bellefonte, PA) warmed by a column heater (Alltech, Derrfield, IL) at 45 °C and detecting by an ultraviolet (UV) detector (UV-2075 Plus, Jasco) at 239 nm [17]. Two high pressure pumps (PU-2080 Plus, Jasco) in series impelled the mobile liquid containing acetonitrile (BDH, Poole, England) gradient from 5% to 50% (v/v) at a fluid flow rate of 0.85 mL/min for 20 min. The entrapment efficiency of SQV in P-T/SQV-SLNs was defined as $[(W_{t,SQV} - W_{s,SQV})/W_{t,SQV}] \times 100\%$, where $W_{t,SQV}$ and $W_{s,SQV}$ were, respectively, the weight of total SQV used in the preparation and the weight of free SQV in the supernatant.

P-T/SQV-SLNs with 0.25 mg/mL were suspended in 4 mL of Dulbecco's phosphate buffered saline (DPBS, Sigma) and activated with 50 μL of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (Sigma) with 1 mM and 50 μL of N-hydroxysuccinimide (sodium salt, Alfa Aesar, Ward Hill, MA) with 1.5 mM at 100 rpm and 4 °C for 1 h. After centrifugation, the pellet was suspended in a solution of 83-14 MAb (Invitrogen, Carlsbad, CA) with 30 μg/mL at 100 rpm and 3 °C for 6 h. The suspension of P-T-MAb/SQV-SLNs was purified with an ultra centrifugal filter device (100 kDa, Millipore, Billerica, MA) at 7000 × *g* for 10 min, frozen, and lyophilized with D-mannitol. QuantiPro™ bicinchoninic acid (BCA) protein assay kit (Sigma) was used to determine the residual quantity of 83-14 MAb after centrifugal filtration. 100 μL of filtrate and 100 μL of working reagent were reacted in a 96-well MicroWell™ polystyrene plate (Nalge Nunc, Rochester, NY) and cultured in a CO_2 incubator (NuAire, Plymouth, MN) with 95% relative humidity and 5% CO_2 at 37 °C for 2 h. An enzyme-linked immunosorbent assay (ELISA) spectrophotometer (Bio-tek, Winooski, VT) at 562 nm was applied to analyze the BCA-incubated solution. After reacting with BCA kit, the optical density of the centrifugal filtrate after grafting 83-14 MAb indicated residual 83-14 MAb in the medium. The grafting efficiency of 83-14 MAb on P-T-MAb/SQV-SLNs was calculated by $[(W_{t,MAb} - W_{s,MAb})/W_{t,MAb}] \times 100\%$, where $W_{t,MAb}$ and $W_{s,MAb}$ were, respectively, the weight of total 83-14 MAb used in the preparation and the weight of free 83-14 MAb in the supernatant. Fig. 1 illustrates the structure of P-T-MAb/SQV-SLNs. Fig. 2 shows the proposed mechanism for binding 83-14 MAb on SLNs.

2.2. Particle size distribution and zeta potential of P-T-MAb/SQV-SLNs

The size distribution and zeta potential of P-T-MAb/SQV-SLNs was obtained by a zetasizer 3000 HS_A with a photon correlation spectroscope and a laser Doppler velocimeter (Malvern, Worcestershire, UK) at 25 °C. 1 mL of suspension containing P-T-MAb/SQV-SLNs with 0.25 mg/mL in 0.1 M tris buffer (Riedel-de Haen) was applied in this analysis.

2.3. Surface structure of P-T-MAb/SQV-SLNs

A field emission scanning electron microscope (FE-SEM, JSM-6330 TF, Jeol, Tokyo, Japan) was used to study the morphology of P-T-MAb/SQV-SLNs. The samples, dehydrated from 20 μL of

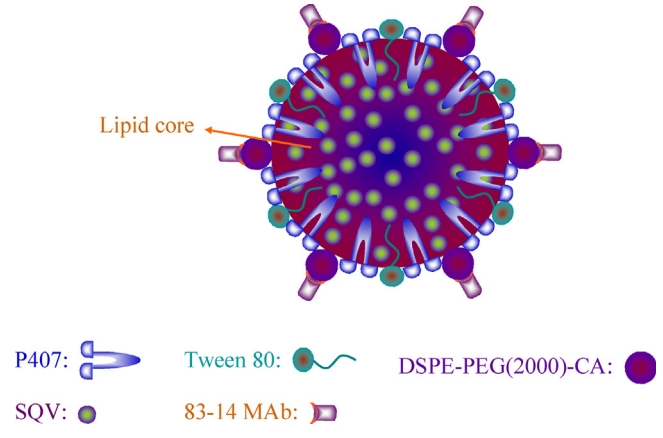

Fig. 1. A schematic representative of a P-T-MAb/SQV-SLN.