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Targeting delivery of saquinavir to the brain using 83-14 monoclonal antibody-grafted solid lipid nanoparticles

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ABSTRACT

83-14 monoclonal antibody (MAb) was modified on solid lipid nanoparticles (SLNs) to improve the brain-targeting delivery of saquinavir (SQV). The endocytosis of 83-14 MAb-grafted SQV-loaded SLNs (83-14 MAb/SQV-SLNs) into human brain-microvasscular endothelial cells (HBMECs) was studied by staining cell nuclei, insulin receptors, and drug carriers. An increase in the weight fraction of palmitic acid in lipid core enhanced the particle size, absolute value of zeta potential, and viability of HBMECs and reduced the entrapment efficiency and release rate of SQV. In addition, an increase in the weight fraction of poloxamer 407 in surfactant layer reduced the particle size, absolute value of zeta potential, phagocytosis by RAW246.7 cells, permeability across the blood–brain barrier (BBB), and uptake by HBMECs and enhanced the viability of HBMECs. Moreover, an increase in the concentration of surface 83-14 MAb enhanced the percentage of surface nitrogen, permeability across the BBB, and uptake by HBMECs and did not significantly vary the viability of HBMECs and phagocytosis by RAW264.7 cells. 83-14 MAb/SQV-SLNs can ameliorate the bioavailability characteristics of SQV, inhibit the lymphatic particle uptake, and promote the transport of SQV into brain endothelia.

1. Introduction

The therapy for acquired immunodeficiency syndrome (AIDS) is an ineluctable challenge to human health care worldwide [1]. AIDS results from infection of human immunodeficiency virus (HIV), which resides mostly in the central nervous system (CNS) and in lymphatic tissues including spleen, lymph node, and intestinal lymph [2]. Several medicines and regimens are currently used to treat AIDS. Saquinavir (SQV), for instance, is a protease inhibitor with biomimetic phenylalanine–proline dipeptide bonds and can efficaciously associate with HIV-1 and HIV-2 to hamper the viral duplication in their later mature stage [3]. However, the bioavailability of SQV is extremely low due mainly to presystemic metabolism by cytochrome P450 3A4 in small intestine and liver [4]. In addition, the efflux pump transporters, such as P-glycoprotein and multidrug resistance protein, restrain the delivery of SQV into the CNS [5–7]. Therefore, an entrapment in appropriate drug carriers can shun the degradation of SQV and improves its brain-targeting release.

Colloidal drug delivery system may be eliminated by phagocytic cells in the reticuloendothelial system (RES) of liver and spleen after administration [8]. A modification with excipient containing polyethylene oxide (PEO) moiety can diminish phagocytosis [9]. For example, surface PEO prevented polymethyl methacrylate (PMMA) particles from capture by the RES organs and preserved the residues of PMMA particles in the heart, gastrointestinal tract, ovary, kidney, muscle, and brain [10]. Poloxamer 407 (P407) with quite a few PEO groups in the structure was approved by the US food and drug administration and applied widely in surface coating and pharmaceutical formulation [11–13]. In fact, P407 could promote the metabolic stability, prolong the blood circulation period, amend the fate of nanocarriers, and adjust the pharmacokinetics [14–16]. In addition, polysorbate 80 (Tween 80) with abundant PEO groups could enhance the quantity of dalargin across the blood–brain barrier (BBB) [17,18]. It has been observed that Tween 80-conjugated colloids deposited on brain microvessel walls [19]. Subsequent endocytosis by brain-microvascular endothelial cells (BMECs) assisted the transport of localized nanoparticles with surface Tween 80 to the brain parenchyma [20,21]. Thus, loaded reagents could be delivered into the CNS via particular release mechanism from Tween 80-coated particles [22,23].

An application of peptide or peptidomimetic monoclonal antibody (MAb) can benefit the drug delivery to the brain by targeting specific endogenous receptors on BMECs [24]. 83-14 MAb, an insulin-like peptidomimetic MAb with molecular weight of 150 kDa, has a strong affinity to brain capillary and can highly bind

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with α-subunit of human insulin receptor (IR) [25]. In addition, the size of 83-14 MAb is similar to that of neuroactive molecule [26]. This size effect may elevate the transport efficiency of 83-14 MAb-grafted particles across the BBB.

The aim of this study is to demonstrate the capability of 83-14 MAb-grafted solid lipid nanoparticles (SLNs) for promoting the delivery of SQV to the brain. We investigated physicochemical properties of SQV-entrapped SLNs with surface 83-14 MAb (83-14 MAb/SQV-SLNs), including the particle size, the zeta potential, the entrapment efficiency of SQV, and the release kinetics. In addition, we examined the following biomedical behavior: the cytotoxicity of 83-14 MAb/SQV-SLNs to human BMECs (HBMECs), the particle phagocytosis by RAW264.7 cells, and the permeability of SQV across the BBB. Moreover, the uptakes of 83-14 MAb/SQV-SLNs by RAW264.7 cells and by HBMECs were analyzed by immunochemical staining.

2. Materials and methods

2.1. Preparation of 83-14 MAb/SQV-SLNs

Dynasan®114 (DYN; Sigma, St. Louis, MO), palmitic acid (PA; Sigma), and SQV (United States Pharmacopeial, Rockville, MD) were dissolved in methanol (J. T. Baker, Phillipsburg, NJ) at 400 rpm and 75 °C. DYN and PA were fixed at 4% (w/v) in methanol and the weight fractions of PA in DYN-PA mixture were 0, 0.33, 0.67, and 1. SQV was controlled at 1.25%, 2.5%, and 5% (w/w) in lipid matrix. 1.25% (w/v) 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (DSPE-PEG (2000)-carboxylic acid; ammonium salt, Avanti Polar Lipid, Alabaster, AL) was added into the organic phase for modifying lipid particle surface. The aqueous phase, containing 0.4% (w/v) cholesteryl hemisuccinate (Sigma) and 1% (w/v) surfactants, was prepared with P407 (Sigma), Tween 80 (Sigma), and sodium dodecyl sulfate (SDS; Sigma) in ultrapure water (Barnstead, Dubuque, IA). The weight fractions of P407 in P407-Tween 80 mixture were 0, 0.5, and 1. 0.1% (w/v) fluorescein isothiocyanate-conjugated dextran 70000 (Sigma) was added into the aqueous phase and incorporated in SQV-entrapped SLNs (SQV-SLNs) for fluorescence. 250 μL of the organic phase was gradually added into 750 μL of the aqueous phase at 400 rpm and 75 °C for 5 min. One aliquot of the emulsified fluid was added into ten aliquots of ultrapure water at 1000 rpm and 3 °C for 15 min. The suspension was filtrated through a filtration paper with pores of 1 μm. The filtrate with solidified SQV-SLNs was centrifuged by a super speed refrigerated centrifuge (AVANTij-25, Beckman Coulter, Palo Alto, CA) at 159,000× *g* and 4 °C for 10 min. The bottom pellet was resuspended in ultrapure water with 2% (w/v) D-mannitol (Sigma), refrigerated in an ultra low temperature freezer (Sanyo, Osaka, Japan) at −80 °C for 30 min, and lyophilized by a freeze dryer (Eyela, Tokyo, Japan) at 2–4 torr and −80 °C for 24 h. SQV in the supernatant was analyzed by a high performance liquid chromatograph (HPLC; Jasco, Tokyo, Japan) with a reverse phase BDS Hypersil C-18 column containing microspheres of 5 μm (Thermo Hypersil-Keystone, Bellefonte, PA) warmed by a column heater (Alltech, Derrfield, IL) at 45 °C and detected by an ultraviolet (UV) detector (UV-2075 Plus, Jasco, Tokyo, Japan) at 239 nm. The mobile phase with acetonitrile (BDH, Poole, England) gradient from 5% to 50% (v/v) for 20 min was driven by two high pressure pumps (PU-2080 Plus, Jasco, Tokyo, Japan) in series for a fluid flow rate of 0.85 mL/min. The entrapment efficiency of SQV, E_{SQV}, was evaluated by $E_{SQV}(\%) = [(W_t - W_s)/W_t] \times 100\%$ [27], where W_t and W_s were, respectively, the total weight of SQV in the preparation and the weight of SQV in the supernatant. Lyophilized SQV-SLNs with 0.25 mg/mL were suspended in 4 mL of Dulbecco's phosphate buffered saline (DPBS, Sigma). 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 were added into 4 mL of suspension of SQV-SLNs at 100 rpm and 4 °C for 1 h. After centrifugation at 159,000× *g* and 4 °C for 10 min, the bottom pellet was resuspended in a solution of 83-14 MAb (Invitrogen, Carlsbad, CA) with 10, 20, and 30 μg/mL at 100 rpm and 3 °C for 6 h. The suspension of 83-14 MAb/SQV-SLNs was dialyzed in an ultra centrifugal filter device (100 kDa, Millipore, Billerica, MA) at 7000× *g* for 10 min, frozen, and lyophilized with D-mannitol. The powders were stored in a refrigerator at 4 °C. The residual 83-14 MAb in the filtrate was analyzed with QuantiPro™ bicinchoninic acid (BCA) protein assay kit (Sigma). Briefly, 100 μL of filtrate and 100 μL of working reagent were mixed in a 96-well MicroWell™ polystyrene plate (Nalge Nunc, Rochester, NY), placed in a humidified CO_2 incubator (NuAire, Plymouth, MN) at 37 °C for 2 h, and quantified by an enzyme-linked immunosorbent assay (ELISA) spectrophotometer (Bio-tek, Winooski, VT) at 562 nm.

2.2. Characterization of 83-14 MAb/SQV-SLNs

2.2.1. Particle size and zeta potential

The cumulant Z-average diameter, *D*, and zeta potential, ζ, of SQV-SLNs were evaluated by a zetasizer 3000 HS_A with a photon correlation spectroscope and a laser Doppler velocimeter (Malvern, Worcestershire, UK) at 25 °C. The concentration of SQV-SLNs in 0.1 M tris buffer (Riedel-de Haen, Seelze, Germany) at pH 7.4 was 0.25 mg/mL. In these analyses, the average diameter and zeta potential used 1 mL and 3 mL suspension, respectively.

2.2.2. SEM morphology

The surface structure of SQV-SLNs was investigated by a field emission scanning electron microscope (FE-SEM, JSM-6330 TF, Jeol, Tokyo, Japan). 20 μL of suspension with 2 mg/mL was dripped down on a cover slide and dehydrated. The samples on cover slide were vacuum-dried, glued with carbon paint, and sputter-coated with platinum with accelerating voltage of 3 kV for 3 min.

2.2.3. TEM morphology

0.2% (w/v) SQV-SLNs in tris buffer was loaded on a carbon-coated 200-mesh copper grid for 2 min. The samples were pretreated with 10% (w/v) phosphotungstic acid solution (Sigma) for 24 h to mark the highly electron-transmissible atoms in SQV-SLNs. A transmission electron microscope (TEM, JEM-1400, Jeol, Tokyo, Japan) was used to examine the particle geometry.

2.2.4. Surface atom

The surface atoms on 83-14 MAb/SQV-SLNs with depth of 10 nm were analyzed by an X-ray photoelectron spectroscope (XPS, Kratos, Kanagawa, Japan) using a vacuum grade of 2×10^{-7} Pa and 300 W and a light bean area of 300 μm × 700 μm. 10 μL of suspension with 2 mg/mL was dripped down on a cover slide of 5 × 5 mm, dehydrated, and vacuum-dried for 15 min before test.

2.3. Release of SQV from 83 to 14 MAb/SQV-SLNs

0.5% (w/v) 83-14 MAb/SQV-SLNs was suspended in DPBS containing 0.05% sodium azide (Sigma) at pH 7.4. A dialysis tubing cellulose membrane (12.4 kDa, Sigma) of 3.3 cm × 30 cm was pretreated with 2% (w/v) sodium bicarbonate (J. T. Baker) for 10 min, purified with ethylene diamine tetra acetic acid (Riedel-de Haën) with 1 mM at pH 8 for 10 min twice, washed with ultra pure water at 4 °C, loaded with 1 mL of the sample, placed in a flask of 100 mL with 50 mL of DPBS at pH 7.4, and shaken in a bath-reciprocal shaker at 80 rpm and 37 °C for 168 h. 100 μL of the liquid sample with dissolved SQV was analyzed by an HPLC–UV system. The fluid medium in the flask was compensated with 100 μL of fresh DPBS immediately at sampling time point. The cumulative released percentage of SQV, P_{SQV}, was evaluated by $P_{SQV}(\%) = Q_c/Q_t \times 100\%$ [28], where Q_c and Q_t were, respectively, the cumulative quantity of SQV in the dissolution buffer and the total quantity of SQV in 83-14 MAb/SQV-SLNs.

2.4. Cytotoxicity assay

The methods for propagating, identifying, and cryopreserving HBMECs (Biocompare, South San Francisco, CA) were described in the literature [29]. Unfrozen HBMECs with a density of 5×10^3 cells/well were seeded into a 96-well plate pretreated with gelatin (Sigma) and incubated with 150 μL of endothelial cell medium (Biocompare) per well in a humidified CO_2 incubator at 37 °C for 12 h. The incubated HBMECs were cultured with 83-14 MAb/SQV-SLNs, SQV-SLNs, or free SQV in a humidified CO_2 incubator at 37 °C for 4 h. The concentration of 83-14 MAb/SQV-SLNs or SQV-SLNs was 0.005%, 0.025%, 0.05%, and 0.1% (w/v) and the concentration of free SQV was 6.25, 12.5, and 25 ppm. 83-14 MAb/SQV-SLNs using 83-14 MAb with 30 μg/mL were applied in the toxicity assessment. The cytotoxicity to HBMECs was assayed with 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT; Biological Industries, Beit Haemek, Israel) and determined by an ELISA spectrophotometer at 450 nm. The cultured HBMECs per well was reacted with 50 μL of XTT mixture containing 2% (v/v) activation solution in a humidified CO_2 incubator at 37 °C for 2 h. The viability of HBMECs, P_{CV}, was evaluated by $P_{CV}(\%) = [(OD_{H,s} - OD_{XTT})/(OD_H - OD_{XTT})] \times 100\%$ [30], where $OD_{H,s}$, OD_H, and OD_{XTT} were, respectively, the optical density of HBMECs incubated with 83-14 MAb/SQV-SLNs, SQV-SLNs, or free SQV, the optical density of HBMECs, and the optical density of XTT.

2.5. Phagocytosis by RAW264.7 cells

Unfrozen RAW264.7 cells (BCRC, Hsin-Chu, Taiwan) with a density of 3.5×10^6 cells/cm^2 were seeded on a gelatin-pretreated T75 tissue culture flask (Corning Costar, Cambridge, MA). RAW264.7 cells were cultured with Dulbecco's modified eagle's medium (Invitrogen), containing 10% (v/v) fetal bovine serum (FBS; Sciencell, Corte Del Cedro Carlsbad, CA) and 1% (v/v) penicillin-streptomycin-glutamine solution (Invitrogen), and expanded in a humidified CO_2 incubator at 37 °C for 4 days. The culture medium was replaced after the initial 8 h and at a rate of every 2 days subsequently. Multiplied RAW264.7 cells were rinsed with 10 mL of DPBS, detached with 4 mL of 0.025% trypsin (Sigma) and diaminoethanetetraacetic acid (Riedel-de Haën) with 0.5 mM, suspended in 4 mL of fresh culture medium, collected in a sterile conical tube (15 mL, BD Falcon, Franklin Lakes, NJ), centrifuged at 150 × *g* for 5 min, immersed in 8 mL of fresh culture medium, and equally distributed into 3 gelatin-pretreated T75 tissue culture flasks. The concentration of