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Entrapment and release of saquinavir using novel cationic solid lipid nanoparticles

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ABSTRACT

Cationic solid lipid nanoparticles (CSLNs) with entrapped saquinavir (SQV) were fabricated by microemulsion method. Here, CSLNs were stabilized by polysorbate 80, and the lipid phase contained cationic stearylamine (SA) and dioctadecyldimethyl ammonium bromide (DODAB) and nonionic Compritol 888 ATO (CA) and cacao butter (CB). Properties of the present pharmaceutical formulations including the entrapment efficiency, the release kinetics, and the distribution of SQV in CSLNs were analyzed. The results indicated that a mixture of SA and DODAB and a mixture of CA and CB were beneficial to the entrapment efficiency of SQV. However, an increase in the content of cationic lipids insignificantly affected the entrapment efficiency of SQV when the weight percentage of SA and DODAB was greater than 1% during emulsification. Also, the rate of SQV released from CSLNs with lipid cores of a mixture of CA and CB was slower than that of pure CB. The temporal variation in the released SQV suggested that the carriers could be sustained delivery systems with no apparent initial burst. Hence, the current CSLNs could carry SQV for the improved medication of individuals infected by human immunodeficiency viruses.

1. Introduction

Saquinavir (SQV) is a protease inhibitor used in the clinical treatment for the acquired immunodeficiency syndrome (AIDS), which is derived from the infection of human immunodeficiency virus (HIV). During the infectious stage, the cleavage position of precursor proteins in HIV can only be recognized by specific viral proteases. Thus, SQV is designed as a peptide similar to the cleavage position for association with the proteases in HIV-1 and HIV-2. Note that $\log D_{oct}$ (the logarithm of the octanol/buffer partition coefficient) of SQV at pH 7.4 is 4.51, indicating that SQV is a rather lipophilic anti-HIV agent. In physiology, the fundamental properties of SQV are 1 h of resident period in blood, 98% of binding to plasma proteins, and 13.2 h of elimination half life for tissue linkage (Lemberg et al., 2002; Strazielle et al., 2005). The main defect of SQV is the low bioavailability of 4–12% (Williams and Sinko, 1999). Besides, SQV belongs to the P-glycoprotein (P-gp) substrate, which is liable to be modulated by the mediation mechanism of P-gp (Lee, 2000; Polli et al., 1999). For transport across the blood–brain barrier, SQV is also inhibited by P-gp (Bachmeier et al., 2005). To improve the absorption rate, dipeptide prodrugs of SQV were designed and demonstrated for shunning the efflux by P-gp (Jain et al., 2007). Except the modification with prodrugs, another efficacious method for enhancement in the bioavailability of SQV is the carrier-mediated system.

The traditional carriers for anti-HIV agents included emulsions, liposomes, polymeric microparticles, and nanoparticles (Kuo and Chung, 2005). For brain-targeting delivery, solid lipid nanoparticles (SLNs) exhibited the typical advantages of nanoparticulate carriers with excellent biocompatibility (Kuo and Kuo, 2008). Moreover, SLNs could extend the half-life of pharmaceuticals in blood and the scale-up feasibility of SLNs was high in practical manufacturing (Bargoni et al., 1998). SLNs were also capable of entrapping antitumor FUdR and carrying FUdR into the central nervous system (Wang et al., 2002). By pharmacokinetic analysis, SLNs were concluded to be a qualified colloidal transporter for the delivery of camptothecin into the brain, the heart, and the reticuloendothelial system (Yang et al., 1999). Furthermore, positively charged carriers were beneficial to the loading of drugs (Kuo, 2005) and to the cellular uptake via electrostatic interactions (Kuo and Lin, 2006). In a study on the binding capacity and the transfection efficiency of genes, cationic SLNs (CSLNs) containing nonionic lipids of Compritol ATO 888 (CA) and cetylpalmitate were prepared (Tabbatt et al., 2004). It was concluded that one-tailed cationic lipids were more toxic than two-tailed cationic lipids such as dioctadecyldimethyl ammonium bromide (DODAB). For CSLNs composed of one-tailed stearylamine (SA), stable complexes of CSLNs, DNA, and

Abbreviations: CA, Compritol 888 ATO; CB, cacao butter; CSLNs, cationic solid lipid nanoparticles; DODAB, dioctadecyldimethyl ammonium bromide; SA, stearylamine; SQV, saquinavir.

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Nomenclature

D	Z-average diameter of CSLNs (nm)
E_e	entrapment efficiency of SQV in CSLNs (%)
$P_{(CA+CB+SQV)/EM}$	weight percentage of nonionic lipids and SQV during emulsification (%)
$P_{CB/(CA+CB)}$	weight percentage of CB in nonionic lipids (%)
$P_{DODAB/EM}$	weight percentage of DODAB during emulsification (%)
$P_{(DODAB+SA)/EM}$	weight percentage of cationic lipids during emulsification (%)
$P_{SA/(DODAB+SA)}$	weight percentage of SA in cationic lipids (%)
$P_{SA/EM}$	weight percentage of SA during emulsification (%)
P_{SQV}	percentage of SQV released from CSLNs (%)
$P_{SQV/(CA+CB+SQV)}$	weight percentage of SQV in nonionic lipids and SQV (%)

Greek letter

ζ	zeta potential of CSLNs (mV)

streptavidin with the binding of biotinylated ligands yielded a high affinity to cellular receptors (Pedersen et al., 2006). Also, the bioavailability and the distribution of anti-psychotic clozapine in the brain were enhanced by the entrapment in CSLNs with cationic SA (Manjunath and Venkateswarlu, 2005). These results implied that CSLNs can be appropriate for carrying lipophilic SQV with ameliorated delivery behavior. For the entrapment of SQV, the internal lipids with complicated biocompatible components were generally required (Müller et al., 2000). Hence, a mixture of CA and cacao butter (CB) (Kim et al., 2005) was employed as the nonionic ingredients of lipid cores in the current CSLNs.

In this study, SQV-entrapping CSLNs with a mixture of CA and CB in the central lipid phase and a mixture of SA and DODAB in the peripheral lipid phase were prepared. Polysorbate 80 in the external layer of CSLNs could prevent the particles from coagulation. The following parameters for the entrapment efficiency of SQV in CSLNs were examined: the ratio of CA to CB, the ratio of SA to DODAB, the content of cationic lipids, and the amount of SQV. Since CA and CB provided various entrapping sites for lipophilic drugs, the distribution of SQV in CSLNs was studied by the nuclear magnetic resonance (NMR). Furthermore, the in vitro dissolution for the release of entrapped SQV from CSLNs was especially analyzed.

2. Materials and methods

2.1. Reagents and chemicals

Dulbecco's phosphate-buffered saline (DPBS), D-mannitol, DODAB, sodium azide, and glutaraldehyde were purchased from Sigma (St. Louis, MO). Deuterium oxide-D was obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA), SQV from United States Pharmacopeial (Rockville, MD), polysorbate 80 from FisherScientific (Fair Lawn, NJ), cacao butter (CB) from OCG Cacao (Whitinsville, MA), CA from Gattefosse S. A. (at Parc des Barbanniers, 92632 Gennevilliers, France), SA from Fluka (Buchs, Switzerland), ethanol from Riedel-de Haën (Seelze, Germany), acetonitrile from BDH (Poole, England), and ultrapure water from Nanopure Infinity Ultrapure System of Barnstead (Dubuque, IA).

2.2. Preparation of SQV-entrapping CSLNs

SQV-entrapping CSLNs with nonionic lipids of CA and CB and cationic lipids of SA and DODAB were fabricated by microemulsion method described previously (Kuo and Su, 2007) with modifications. Briefly, based on the overall microemulsified fluid, 4% (w/w) nonionic lipids and SQV was mixed with cationic lipids under magnetic stirring at 75 °C. 8% (w/w) polysorbate 80 and 7.5% (w/w) ethanol were dissolved in ultrapure water and preheated at 75 °C. The compositions of the primary microemulsions are summarized in Table 1. The surfactant solution was mixed with the melted lipids and SQV at 500 rpm and 75 °C for 3 min. One aliquot of the microemulsified liquid was added into 10 aliquots of ultrapure water at 500 rpm and 3 °C for 20 min. The suspension containing newly formed CSLNs was filtrated by a filtration paper with pores of 1 μm. SQV-entrapping CSLNs in the filtrate were separated by a refrigerated superspeed centrifuge (CP80MX, Hitachi Koki, Tokyo, Japan) with a rotor of P40ST-1591 in a conical centrifugal microtube (Falcon, Franklin Lakes, NJ) at 236,500 × g for 30 min. Bottom pellet containing CSLNs of 60 mg was resuspended in ultrapure water of 10 mL with 2% (w/v) D-mannitol, refrigerated at 4 °C for 30 min, at −20 °C in a low temperature freezer (Frigidaire, Augusta, GA) for 30 min, and at −80 °C in an ultralow temperature freezer (Sanyo, Osaka, Japan) for 30 min, and lyophilized at −80 °C and 4 Pa (Eyela, Tokyo, Japan) over 24 h to obtain powder products. The existence of CSLNs in the supernatant was checked by a zetasizer 3000 HS_A (Malvern, Worcestershire, UK) for confirmation of the completion in centrifugal separation. The supernatant was also applied to the estimation of the entrapment efficiency.

Several formulations of CSLNs were obtained by alteration in the ratio of CA to CB, the weight percentage of SA and DODAB, the ratio of SA to DODAB, and the weight percentage of SQV. For the high resolution proton nuclear magnetic resonance (^{1}H NMR) analysis, CSLNs were prepared without ethanol and D-mannitol.

2.3. Entrapment efficiency of SQV

The wavelength of UV absorbance for SQV was scanned by a UV microplate spectrophotometer (Bio-Tek, Winooski, VT) in the range of 200–400 nm and the maximal absorbance was 239 nm, which was consistent with the literature result (Glynn and Yazdanian, 1998). The amount of SQV in the supernatant after centrifugation was evaluated by a high performance liquid chromatography (Jasco, Tokyo, Japan) with a UV–vis spectrophotometer (UV-2075 Plus, Jasco, Tokyo, Japan) at 239 nm. A reverse phase BDS Hypersil C18 column containing particles of 5 μm (Thermo Hypersil-Keystone, Bellefonte, PA) was warmed by a column heater (Alltech, Derrfield, IL) at 45 °C. Two high-pressure pumps (PU-2080 Plus, Jasco, Tokyo, Japan) in series were applied to the mobile phase containing ultrapure water and acetonitrile with the gradient from 20 to 50% in 20 min with a fluid flow rate of 0.85 mL/min. The retention time of SQV was about 8.7 min and the R-squared value for the calibration of SQV at 239 nm was about 0.99. The entrapment efficiency, E_e, was calculated by:

$$E_e = \frac{\text{total weight of SQV} - \text{weight of SQV in supernatant}}{\text{total weight of SQV}} \times 100\%$$

2.4. Characterization of SQV-entrapping CSLNs

2.4.1. Particle size and morphology

The particle size distribution, the cumulant Z-average diameter, and the zeta potential of SQV-entrapping CSLNs were obtained by a zetasizer 3000 HS_A with a photon correlation spectroscope and a Laser Doppler velocimeter (Malvern, Worcestershire, UK) at 25 °C. CSLNs in ultrapure water with a concentration of 2 mg/mL were employed in the detection. The duration of the detection was 120 s for the particle size distribution and the average diameter and was 20 s for the zeta potential. The assumptions for the calcu-