Colloids and Surfaces B: Biointerfaces 83 (2011) 299–306

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Physicochemical properties of nevirapine-loaded solid lipid nanoparticles and nanostructured lipid carriers

Yung-Chih Kuo*, Jui-Fang Chung

Department of Chemical Engineering, National Chung Cheng University, Chia-Yi, 62102, Taiwan, ROC

ARTICLE INFO

Article history:
Received 25 October 2010
Received in revised form
23 November 2010
Accepted 28 November 2010
Available online 4 December 2010

Keywords:
Solid lipid nanoparticle
Nanostructured lipid carrier
Surface charge
Human serum albumin
Nevirapine

ABSTRACT

Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) coated with human serum albumin (HSA) were fabricated for formulating nevirapine (NVP). Here, NLCs contained low-melting-point oleic acid (OA) in the internal lipid phase. The results revealed that the two nanoparticles were uniformly distributed with the average diameter ranging from 145 to 180 nm. The surface HSA neutralized the positive charge of dimethyldioctadecyl ammonium bromide (DODAB) on SLNs and NLCs and reduced their zeta potential. In a fixed ratio of solid lipids, SLNs entrapped more NVP than NLCs. The incorporation of OA also reduced the thermal resistance of NLCs and accelerated the release of NVP from the nanocarriers. When incubated with DODAB-stabilized SLNs, the viability of human brain-microvascular endothelial cells (HBMECs) reduced. However, the surface HSA increased the viability of HBMECs about 10% when the concentration of SLNs was higher than 0.8 mg/mL. HSA-grafted SLNs and NLCs can be effective formulations in the delivery of NVP for viral therapy.

1. Introduction

Nevirapine (NVP), a non-nucleoside reverse transcriptase inhibitor, is one of the most prescribed antiretroviral drugs for reducing the morbidity and mortality related to the infection of human immunodeficiency virus (HIV) [1]. NVP has been widely applied as a prophylaxis dose against mother-to-child HIV transmission in developing countries [2]. In combination therapy, NVP was used in the initial regimen to reduce impacts on the clinical implications of drug resistance [3]. From pharmacological point of view, NVP can bind to the vicinity of the active site in reverse transcriptase and weaken the catalytic ability of the viral enzyme to transcribe ribonucleic acid. However, NVP can be metabolized by CYP3A and produce phenolic and electrophilic derivatives, which may form covalent adducts of deoxyribonucleic acid and induce mutagenicity and hepatocarcinogenicity [4]. In addition to carcinogenicity, the common NVP-associated side effects include hepatitis, hepatic failure, Stevens-Johnson syndrome, severe rash, eosinophilia, granulocytopenia, lymphadenopathy, and renal dysfunction [5]. The colloidal delivery system of NVP using lipid vehicles may shun the hepatic metabolism and adverse reactions.

The application of solid lipid pellets to administer drugs via peroral route was developed in the early 1990s [6]. The further evolution yielded biomimetic solid lipid nanoparticles (SLNs), which combined the advantages of polymer, lipid, and liposome, and might reduce the burst release of drug [7,8]. Therefore, SLNs were regarded as an appropriate formulation for intravenous (i.v.) administration [9]. After i.v. administration to rodents, SLNs prolonged drug circulation and enhanced the delivery of doxorubicin and FUDR prodrug to the brain [10,11]. After intraduodenal administration to rats, the transport of SLNs to the lymph and blood plasma was evidenced by photon correlation spectroscopy, transmission electron microscopy, and gamma-counting [12]. In addition, colloidal particles generally accumulated in the reticuloendothelial system (RES). SLNs enhanced the uptake of camptothecin in the reticuloendothelial cells-containing organs and extended the mean residence times in the brain, heart, and RES organs [13]. Moreover, the surface properties of nanoparticles could alter their uptake in the RES organs [14]. Nanostructured lipid carriers (NLCs) possessed a portion of liquefied cores with a low-melting-point component, in general. Therefore, the properties of NLCs as drug carriers could be very different from SLNs.

Human serum albumin (HSA) is a promising biomacromolecule and draws a great attention in both fundamental and applied medication due to its biodegradability, nontoxicity, nonimmuno-

Abbreviations: CA, Compritol 888 ATO; HBMEC, human brain-microvascular endothelial cell; HSA, human serum albumin; HSA/NVP-NLC, NVP-loaded NLC with surface HSA; HSA/NVP-SLN, NVP-loaded SLN with surface HSA; NLC, nanostructured lipid carrier; NVP, nevirapine; NVP-NLC, NVP-loaded NLC; NVP-SLN, NVP-loaded SLN; OA, oleic acid; SA, steric acid; SLN, solid lipid nanoparticle.

* Corresponding author. Tel.: +886 5 272 0411x33459; fax: +886 5 272 1206.
E-mail address: chmyck@ccu.edu.tw (Y.-C. Kuo).

0927-7765/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfb.2010.11.037

Nomenclature

$C_{particle}$	concentration of particle in medium (mg/mL)
D	average particle diameter (nm)
E_e	entrapment efficiency of NVP (%)
$P_{CA/(SA+CA)}$	weight percentage of CA in SA and CA (%)
P_{CV}	viability of HBMECs (%)
P_{NVP}	cumulative percentage of NVP released (%)
t	release time (h)
ζ	zeta potential (mV)

genicity, and regulatory function [15]. For example, HSA could stabilize the ingredients in vaccines and modify the surfaces of medical devices [16,17]. In addition, the antioxidant property of HSA was beneficial to pharmaceutical formulations [18,19]. The ligand-binding capability also provided the opportunity of HSA to construct possible innovated structure for therapeutic carriers [20]. In fact, HSA were used in fabricating Albunex and ABI-007 for clinical purpose [21,22].

The aim of this study is to investigate the physicochemical properties of HSA-grafted SLNs and NLCs. NVP is an important drug in treating the HIV-infected individuals. The entrapment of NVP in lipid phase can enhance its biocompatibility. Therefore, NVP was entrapped in SLNs and NLCs. The biocompatibility and the release kinetics of NVP from the two nanoparticulate formulations were investigated.

2. Materials and methods

2.1. Reagents and chemicals

Steric acid (SA), oleic acid (OA), dimethyldioctadecyl ammonium bromide (DODAB), D-trehalose dehydrate, HSA, Dulbecco's phosphate buffered saline (DPBS), sodium azide, 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), and phosphotungstic acid solution were purchased from Sigma (St. Louis, MO). Ethylene-diamine-tetra-acetic acid (EDTA), 1-butanol, and tris (hydroxymethyl) aminomethane (Tris) were obtained from Riedel-de Haen (Seelze, Germany). 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Acros (Morris, NJ). Human brain-microvascular endothelial cells (HBMECs) and endothelial cell medium (ECM) were obtained from Biocompare (South San Francisco, CA). Compritol 888 ATO (CA) was purchased from Gattefosse S. A. (Gennevilliers, France), Tween 80 from FisherScientific (Fair Lawn, NJ), NVP from ChemPacific (Blatimore, MD), acetonitrile from BDH (Poole, England), polycarbonate membrane from Millipore (Bedford, MA), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[carboxy(polyethylene glycol)-2000] (DSPE-PEG(2000) carboxylic acid) from Avanti Polar Lipid (Alabaster, AL), hydroxylamine hydrochloride from Alfa Aesar (Karlsruhe, Germany), and ultrapure water from Nanopure Infinity Ultrapure System of Barnstead (Dubuque, IA).

2.2. Preparation of NVP-SLNs, NVP-NLCs, HSA/NVP-SLNs and HSA/NVP-NLCs

NVP-loaded SLNs (NVP-SLNs) containing CA, SA, DODAB, and Tween 80 were fabricated by microemulsion method described previously with modifications [23]. CA, SA, and NVP were mixed as a lipid phase at 75 °C. NVP was controlled at 2.5% (w/w) the weight of CA and SA. The weight ratios of CA in CA and SA were 0, 0.25, 0.5, 0.75, and 1. The lipid weight in the microemulsion was 4% (w/w). 1.8% (w/w) DODAB, 1% (w/w) Tween 80, 0.2% (w/w) lecithin, and 5% (w/w) 1-butanol were dissolved in ultrapure water under magnetic stirring and preheated at 75 °C. In the preparation of NVP-loaded NLCs (NVP-NLCs), OA was added and the weight percentage of OA in the internal lipids was 25%. Table 1 summarizes the compositions of lipids and surfactants in the microemulsion. The aqueous solution containing surfactants was mixed with the melted lipids at 400 rpm and 75 °C for 3 min. One aliquot of the microemulsion was added into 10 aliquots of ultrapure water at 500 rpm and 3 °C for 15 min. The fluid containing nanoparticles was filtrated by a filtration paper with pores of 1 μm. NVP-SLNs and NVP-NLCs in the filtrate were centrifuged by a superspeed refrigerated centrifuge (AVANTij-25, Beckman Coulter, Palo Alto, CA) at 159000 × *g* and 4 °C for 30 min. The bottom pellet was resuspended in ultrapure water containing 2% (w/v) D-trehalose, refrigerated in an ultralow temperature freezer (Sanyo, Osaka, Japan) at −80 °C for 30 min, and lyophilized at −80 °C and 4 Pa (Eyela, Tokyo, Japan) over 24 h. The supernatant was analyzed 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 heater (Alltech, Derrfield, IL) at 35 °C. An ultraviolet (UV) detector (Jasco, Tokyo, Japan) at 234 nm followed the HPLC. The entrapment efficiency of NVP, E_e, was calculated by E_e = [(total weight of NVP − weight of NVP in supernatant)/(total weight of NVP)] × 100% [24].

To modified HSA, 0.1% (w/w) lecithin was replaced by 0.1% (w/w) DSPE-PEG(2000) carboxylic acid in the surfactant phase. EDC and NHS with a molar ratio of 2:5 (2 mM:5 mM) were mixed with the suspension of DSPE-PEG(2000) carboxylic acid-coated NVP-SLNs and NVP-NLCs at 80 rpm and 25 °C for 1 h. After centrifugation at 159000 × *g* and 4 °C for 30 min, the pellet was resuspended in a solution containing HSA at 80 rpm and 25 °C for 2 h. The concentrations of HSA were 0.05, 0.1, 0.5, and 1 mg/mL. 10 mL hydroxylamine was added into the suspension to terminate the crosslinking. NVP-SLNs with surface HSA (HSA/NVP-SLNs) and NVP-NLCs with surface HSA (HSA/NVP-NLCs) were purified using a dialysis tube of 100 kDa [25]. Fig. 1 illustrates the representative structure of the particles.

2.3. Particle size and zeta potential

The particle size distribution, cumulant Z-average diameter (D), and zeta potential (ζ) of NVP-SLNs, NVP-NLCs, HSA/NVP-SLNs, and

Table 1
Weight percentage of the ingredients in microemulsion for preparing the lipid nanoparticles.

	NVP-SLNs	NVP-NLCs	HSA/NVP-SLNs	HSA/NVP-NLCs
Lipid	SA and CA: 4% NVP: 0.1%	OA: 1% SA and CA: 3% NVP: 0.1%	SA and CA: 4% NVP: 0.1%	OA: 1% SA and CA: 3% NVP: 0.1%
Surfactant	DODAB: 1.8% Tween 80: 1% Lecithin: 0.2%	DODAB: 1.8% Tween 80: 1% Lecithin: 0.2%	DODAB: 1.8% Tween 80: 1% Lecithin: 0.1% DSPE-PEG(2000) carboxylic acid: 0.1%	DODAB: 1.8% Tween 80: 1% Lecithin: 0.1% DSPE-PEG(2000) carboxylic acid: 0.1%
Cosurfactant	1-butanol: 5%	1-butanol: 5%	1-butanol: 5%	1-butanol: 5%