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Polyethyleneimine/poly-(γ-glutamic acid)/poly(lactide-*co*-glycolide) nanoparticles for loading and releasing antiretroviral drug

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ABSTRACT

Nanoparticles (NPs) with ternary components of polyethyleneimine (PEI), poly-(γ-glutamic acid) (γ-PGA), and poly(lactide-*co*-glycolide) (PLGA) were applied to carry and release saquinavir (SQV). Hydrophobic SQV was encapsulated in the particle core composed of PLGA to form SQV-PLGA NPs, and the surface of SQV-PLGA NPs was grafted successively with hydrophilic γ-PGA and PEI (PEI/γ-PGA/SQV-PLGA NPs). The morphological images revealed that PEI/γ-PGA/SQV-PLGA NPs were spheroid-like, in general. An increase in the concentration of didecyl dimethylammonium bromide and a reduction in the dose of SQV enhanced the entrapment efficiency of SQV in PLGA NPs. In addition, an increment in the molecular weight of γ-PGA reduced the grafting efficiency of PEI on γ-PGA/SQV-PLGA NPs. An increase in the weight percentage of PEI enhanced the average particle diameter. However, the grafting efficiency of PEI on γ-PGA/SQV-PLGA NPs and the dissolution rate of SQV from PEI/γ-PGA/SQV-PLGA NPs reduced when the weight percentage of PEI increased. PEI/γ-PGA/SQV-PLGA NPs are an innovative drug delivery system and can be used for antiretroviral trials.

1. Introduction

Saquinavir (SQV) is a typical protease inhibitor for the medication of acquired immunodeficiency syndrome [1]. The major drawback of SQV in clinical practice is its low permeability across the blood–brain barrier (BBB) to suppress human immunodeficiency virus residing in the central nervous system (CNS) [2]. In normal physiology, the BBB exhibits a high transendothelial electrical resistance to restrain most substances, including antiretroviral drugs, from passing into the brain via paracellular route [3,4]. Therefore, the transcellular transport becomes the main pathway to deliver SQV into the CNS. In addition, the key component of the BBB is brain-microvascular endothelial cells (BMECs), which possess sialic acid residues of acidic glycoprotein to produce anionic domain on the cell luminal front (blood-encountering surface) [5]. Thus, BMECs tend to attract positively charged entities via electrostatic interaction, and a conjugation with cationic colloids may enhance endocytosis and raise the antiretroviral efficiency of SQV [6–10].

Poly(lactide-*co*-glycolide) (PLGA) is a degradable biomaterial used in a variety of medical devices and is appropriate for loading hydrophobic drugs such as SQV [11]. In addition, poly-(γ-glutamic acid) (γ-PGA) is a biodegradable macromolecule developed rapidly in the last two decades [12]. An incorporation of γ-PGA into antigenic formulation, influenza vaccine, and gene transfection carrier showed improved pharmaceutical efficacies [13–16]. A modification of γ-PGA could also promote the surface hydrophilic characteristics and sustain the delivery of hydrophilic drug [17]. Moreover, polyethyleneimine (PEI) is a branched biopolymer with a high density of amine groups. The primary amines of PEI can effectively absorb protons to produce positive charge, rendering a strong affinity to cells [18–20]. PEI-grafted scaffolds were concluded to be promising in chondrocyte recruitment and cartilage tissue engineering [21]. In enzymatic response, PEI can enhance the stability of proteins by chelating metal ions and by shunning the oxidation of sulfhydryl groups.

Various kinds of nanoparticles (NPs) have been widely used in carrying and transporting drugs [22]. For example, polymeric and solid lipid NPs were capable of delivering antiretroviral agents across the BBB [23,24]. In addition, the delivery of NP-mediated anticancer pharmaceuticals to malignant tumor could be obtained through active targeting pathways [25,26]. In these drug delivery systems, the use of biocompatible NPs is regarded as a critical issue.

Abbreviations: DDAB, didecyl dimethylammonium bromide; NP, nanoparticle; PEI, polyethyleneimine; PEI/γ-PGA/SQV-PLGA, PEI-grafted γ-PGA/SQV-PLGA; PLGA, poly(lactide-*co*-glycolide); SQV, saquinavir; SQV-PLGA, SQV-encapsulated PLGA; γ-PGA, poly-(γ-glutamic acid); γ-PGA/SQV-PLGA, γ-PGA-grafted SQV-PLGA.

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Nomenclature

C_{SQV}	concentration of SQV in organic phase (μM)
EE_{SQV}	entrapment efficiency of SQV in PEI/γ-PGA/SQV-PLGA (%)
GE_{PEI}	grafting efficiency of PEI on γ-PGA/SQV-PLGA (%)
$MW_{γ\text{-}PGA}$	average molecular weight of γ-PGA (kDa)
P_{DDAB}	weight percentage of DDAB in microemulsion (%)
P_{PEI}	weight percentage of PEI in micromulsion (%)
P_{SQV}	cumulative percentage of SQV released from PEI/γ-PGA/SQV-PLGA (%)
t	dissolution time (day)

The combination of PEI, γ-PGA, and PLGA can be appropriate for formulating drugs in polymeric NPs with high biocompatibility.

The aim of this study is to develop NPs comprising SQV-PLGA in the core with surface γ-PGA and PEI (PEI/γ-PGA/SQV-PLGA NPs) as a drug delivery system. Hydrophilic γ-PGA and charged PEI grafted on the hydrophobic SQV-PLGA NPs can diversify and improve the dissolution characteristics of SQV from PEI/γ-PGA/SQV-PLGA NPs. The particle size distribution, particle morphology, grafting efficiency of PEI, entrapment efficiency of SQV, and release kinetics of SQV were investigated.

2. Materials and methods

2.1. Reagents and chemicals

Propylene carbonate (PC), didecyl dimethylammonium bromide (DDAB), D-mannitol, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), PEI (average MW 25,000), phosphotungstic acid solution, Dulbecco's phosphate buffered saline (DPBS), and sodium azide were purchased from Sigma (St. Louis, MO). N-hydroxysuccinimide (NHS) was obtained from Acros (Morris, NJ), *Bacillus licheniformis* ATCC 9945a from BCRC (Hsin-Chu, Taiwan), PLGA (average MW 100,000) from Purac (Bingen, Germany), tris (hydroxymethyl) aminomethane (Tris) from Riedel-de Haen (Seelze, Germany), SQV ($C_{38}H_{50}N_6O_5$, MW 670.841) from United States Pharmacopeial (Rockville, MD), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (DSPE-PEG(2000) carboxylic acid) from Avanti Polar Lipid (Alabaster, AL), dimethyl sulfoxide (DMSO) from J.T. Baker (Phillipsburg, NJ), acetonitrile from BDH (Poole, England), acetone from Mallinckrodt Baker (Hazelwood, MO), and ultrapure water from Nanopure Infinity Ultrapure System of Barnstead (Dubuque, IA).

2.2. Thermal decomposition

γ-PGA was synthesized by fermentation of *B. licheniformis* ATCC 9945a. The freshly prepared γ-PGA was treated thermally for obtaining different molecular weight products. 100 mg γ-PGA was dissolved in 100 mL of ultrapure water, degraded in a batch reactor (Chan-Ja Automatic, Tainan, Taiwan) at 150 rpm and 150 °C for 1, 3, and 5 h, and centrifuged at 20,000 × *g* and 4 °C for 30 min. The bottom pellet was gathered. The viscosity average molecular weight of γ-PGA, M_v, was determined by the following Mark–Houwink–Sakurada equation [27]: $[\eta] = 4.1 \times 10^{-5} M_v^{0.94}$, where $[\eta]$ is the intrinsic viscosity. $[\eta]$ can be estimated by $[\eta] = \lim_{C\to 0}(\eta_{sp}/C) = \lim_{C\to 0}(\ln \eta_r/C)$, where $\eta_{sp} = (\eta - \eta_{s,\gamma})/\eta_{s,\gamma}$ and $\eta_r = \eta/\eta_{s,\gamma}$ [28,29]. In these expressions, η_{sp}, η_r, η, $\eta_{s,\gamma}$, and C are the specific viscosity, the relative viscosity, the viscosity of water, the viscosity of the γ-PGA solution, and the concentration of γ-PGA, respectively. $\eta_{s,\gamma}$ of the solution containing 0.01–0.05% (w/v) γ-PGA was obtained by an MCR 500 rheometer (Anton Paar, Graz, Austria) at a shear rate of 10 s^{-1} and analyzed by US 200 software. An extrapolation on the data of η_{sp} or $\ln \eta_r$ to C approaching zero led to $[\eta]$. Thus, M_v was evaluated from the Mark–Houwink–Sakurada equation.

2.3. Preparation of SQV-PLGA NPs

PLGA NPs entrapping SQV (SQV-PLGA NPs) were prepared by an emulsification-diffusion method. 20 mg PLGA and 0.67–2 mg (50–150 μM) SQV were dissolved in 20 mL of PC at 400 rpm and 25 °C for 1 h. 12–48 mg DDAB and 0.4–1.6 mg DSPE-PEG(2000)-carboxylic acid (maintained at 3.34% (w/w) DDAB) were mixed in 40 mL of ultrapure water at 400 rpm and 25 °C for 1 h. The two solutions were emulsified by a homogenizer (PT 2100, Kinematica AG, Lucerne, Switzerland) at 30,000 rpm and 25 °C for 10 min. 140 mL of ultrapure water was added into the microemulsion at 1000 rpm and 25 °C for 1 h. SQV-PLGA NPs were dialyzed in a dialysis bag of 12.4 kDa for 1 h. The dialysate was analyzed by a high performance liquid chromatography (HPLC, Jasco, Tokyo, Japan) with a UV–visible detector (UV-2075 Plus, Jasco, Tokyo, Japan) at 239 nm. The mobile phase containing a gradient of acetonitrile from 5% to 45% was driven by two high-pressure pumps (PU-2080 Plus, Jasco, Tokyo, Japan) in series with a flow rate of 0.85 mL/min for 20 min. The entrapment efficiency of SQV in PLGA NPs, EE_{SQV}, was defined as [30]

$$EE_{SQV} = \left[\frac{\text{weight of total SQV} - \text{weight of free SQV}}{\text{weight of total SQV}}\right] \times 100\%.$$

The dialyzed SQV-PLGA NPs were filtrated. 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 resuspended in ultrapure water containing 2% (w/v) D-mannitol, frozen in an ultralow 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 over 24 h. The powders of SQV-PLGA NPs were stored at 4 °C.

2.4. Fabrication of PEI/γ-PGA/SQV-PLGA NPs

0.01% (w/v) SQV-PLGA NPs, 0.096% (w/v) EDC, and 0.023% (w/v) NHS were mixed at 150 rpm and 25 °C for 4 h [31]. These SQV-PLGA NPs were crosslinked with 0.005% (w/v) γ-PGA at 150 rpm and 25 °C for 4 h. The suspension was centrifuged at 159,000 × *g* and 4 °C for 1 h. γ-PGA/SQV-PLGA NPs in the pellet were collected and dried at 50 °C. Furthermore, 0.01% (w/v) γ-PGA/SQV-PLGA NPs, 0.096% (w/v) EDC, and 0.023% (w/v) NHS were mixed at 150 rpm and 25 °C for 4 h. The activated γ-PGA/SQV-PLGA NPs were grafted with 0.01–0.04% (w/v) PEI at 150 rpm and 25 °C for 4 h. The suspension was centrifuged at 159,000 × *g* and 4 °C for 1 h. PEI/γ-PGA/SQV-PLGA NPs in the pellet were collected and dried at 50 °C. Fig. 1 illustrates the composite structure of PEI/γ-PGA/SQV-PLGA NP. The quantity of free PEI in the supernatant was determined by the HPLC followed with the UV detector at 201 nm. The grafting efficiency of PEI on γ-PGA/SQV-PLGA NPs, GE_{PEI}, was calculated by [32]

$$GE_{PEI} = \left[\frac{\text{weight of total PEI} - \text{weight of free PEI}}{\text{weight of total PEI}}\right] \times 100\% = \left[1 - \frac{\text{weight of free PEI}}{\text{weight of total PEI}}\right] \times 100\%.$$

2.5. Particle size distribution and morphology

The particle size distribution of PEI/γ-PGA/SQV-PLGA NPs was analyzed by a zetasizer 3000 HS_A (Malvern, Worcestershire, UK)