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Electromagnetic interference in the permeability of saquinavir across the blood–brain barrier using nanoparticulate carriers

Yung-Chih Kuo *, Chan-Ying Kuo

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

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Abstract

Transport of antiretroviral agents across the blood–brain barrier (BBB) is of key importance to the treatment for the acquired immunodeficiency syndrome (AIDS). In this study, impact of exposure to electromagnetic field (EMF) on the permeability of saquinavir (SQV) across BBB was investigated. The *in vitro* BBB model was based on human brain-microvascular endothelial cells (HBMEC), and the concentration of SQV in receiver chamber of the transport system was evaluated. Polybutylcyanoacrylate (PBCA), methylmethacrylate-sulfopropylmethacrylate (MMA-SPM), and solid lipid nanoparticle (SLN) were employed as carriers for the delivery systems. Cytotoxicity of SLN decreased as content of cacao butter increased. Power of 5 mV was apposite for the study on HBMEC without obvious apoptosis. Square wave produced greater permeability than sine and triangle waves. The carrier order on permeability of SQV across HBMEC monolayer under exposure to EMF was SLN > PBCA > MMA-SPM. Also, a larger frequency, modulation or depth of amplitude modulation (AM), or modulation or deviation of frequency modulation (FM) yielded a greater permeability. Besides, enhancement of permeability by AM wave was more significant than that by FM wave. Transport behavior of SQV across BBB was strongly influenced by the combination of nanoparticulate PBCA, MMA-SPM, and SLN with EMF exposure. This combination would be beneficial to the clinical application to the therapy of AIDS and other brain-related diseases.

Keywords: Blood–brain barrier; Electromagnetic field; Polybutylcyanoacrylate; Methylmethacrylate-sulfopropylmethacrylate; Solid lipid nanoparticle; Saquinavir

1. Introduction

Blood–brain barrier (BBB) is an important tissue site, providing a neurogenic environment for homeostasis of the central nervous system (CNS) against intense fluctuations in the level of substances in the circulatory system (Clark, 1999; Edwards, 2001). Brain-microvascular endothelial cells (BMEC) play a crucial role in the BBB characteristics. For example, tight junction (TJ) among BMEC is a key structure in avoiding dramatic influences of neurotransmitters and hormones on the cerebrum. Also, P-glycoprotein in BMEC membrane generates several essential pharmacological functions for drug carriage and expulsion (Gelperina et al., 2002).

Transport of anti-human immunodeficiency virus (HIV) agents across BBB is a prerequisite for the treatment of the acquired immunodeficiency syndrome (AIDS) (Glynn and Yazdanian, 1998) because HIV is replicated in the brain of HIV-infected individuals (Pang et al., 1990). Saquinavir (SQV) is a protease inhibitor for reducing enzymatic activity and inducing immature filial generation of HIV. That is, SQV medication generates noninfectious virus to obtain curative effect on the AIDS therapy. Although SQV is hydrophobic with log (octanol/buffer partition coefficient) of 4.51 and possesses high bioavailability via oral administration, the BBB permeability of SQV is very low (Lemberg et al., 2002; Li and Chan, 1999; Strazielle and Ghersi-Egea, 2005).

For drug delivery into CNS, pharmaceutical strategies, such as unfolding of TJ hiatus, utilization of prodrugs, and mediation by carrier systems, have been developed. Since pharmaceuticals are normally ionogenic through absorption or dissociation of protons in plasma, charged colloids become beneficial to the carrier-medicated delivery. Polybutylcyanoacrylate (PBCA) and methylmethacrylate-sulfopropylmethacrylate (MMA-SPM) were efficacious nanoparticle (NP) carriers for meliorating permeability of zidovudine and lamivudine across BBB (Kuo and Chen, 2006). Besides, concentrations of doxorubicin (Fundrao et al., 2000) and camptothecin (Yang et al., 1999) in rat brain tissue were obviously increased by

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

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entrapment in solid lipid nanoparticles (SLN). The three particulate formulations were capable of brain-targeted delivery and dosage reduction.

Exposure to electromagnetic field (EMF) could cause significant alteration in the BBB behavior. In a study on the transport of D-mannitol across BBB, it was observed that a higher power generally yielded larger permeability, and pulse wave was more effective in the permeability enhancement than continuous wave (Oscar and Hawkins, 1977). Also, intensity of sodium fluorescein in brain tissue exposed to EMF for 30 min was larger than that without EMF treatment (Williams et al., 1984a). Twofold increase in BBB permeability of sucrose was obtained after exposure to EMF of 1.8 GHz over 4 days (Schirmacher et al., 2000). For the thermal effect of EMF on BBB permeability, permeability enhancement was accompanied with an increase of 0.4 °C in brain tissue (Albert and Kerns, 1981). However, thermal effect of EMF might not be the predominant reason for permeability enhancement because the temperature increase was within the variation of 1 °C in daily rhythm. In a study on the penetration of horseradish peroxidase across BBB, horseradish peroxidase was observed in the outer zone of BMEC after exposure to EMF for 2 h, and regular transport was retrieved after termination of EMF for 1–2 h, indicating temporary alteration in BBB behavior by EMF exposure (Williams et al., 1984b).

In the present study, permeability of SQV across BBB under exposure to EMF was investigated *in vitro*. Here, BBB model was based on human BMEC (HBMEC), and SQV was incorporated with PBCA, MMA-SPM, and SLN. Effects of systematic parameters of EMF, including power, wave types, frequency, modulation and depth of amplitude modulation (AM) wave, and modulation and deviation of frequency modulation (FM) wave, were especially examined.

2. Materials and methods

2.1. Reagents and chemicals

Ammonim persulfate (APS), mannitol, Dulbecco's phosphate buffered saline (DPBS), L-α-phospatidylcholine (PC), cholesteryl hemisuccinate, taurocholate, D-trehalose, fluorescein isothiocyante (FITC)-conjugated dextrain, gelatin, trypsin–EDTA, rat-tail collagen, human fibronectin, 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), anti-human von Willebrand factor VIII, anti-rabbit IgG with FITC conjugate, and [^{14}C]sucrose were purchased from Sigma (St. Louis, MO). Sulfopropyl methacrylate and docosanoic acid were obtained from Aldrich (Milwaukee, WI). Methyl methacrylate (MMA) and dextran 70,000 were purchased from Fluka Biochemika (Buchs, Switzerland). SQV was obtained from United States Pharmacopeial (Rockville, MD), butylcyanoacrylate (BCA) from Sicomet (Sichel Werk, Germany), polysorbate 80 from FisherScientific (Fair Lamn, NJ), acetonitrile from BDH (Poole, England), cacao butter (CB) from OCG Cacao (Whitinsville, MA), endothelial cell medium (ECM) from Biocompare (South San Francisco, CA), polycarbonate membrane from Millipore (Bedford, MA), Triton-X 100 from Acros (Geel, Belgium), methanol from Mallinckrodt Baker (Phillipsburg, NJ), and ultrapure water from Nanopure Infinity Ultrapure System of Barnstead (Dubuque, IA).

2.2. Preparation of SQV-incorporated carriers

Synthesis processes of PBCA and MMA-SPM nanoparticles and loading procedures of SQV were described previously (Kuo, 2005) with minor modifications. Briefly, BCA was polymerized in an acidic medium containing dextran 70,000 at 25 °C. MMA and SPM were copolymerized in the presence of APS at 78 °C over 24 h. PBCA and MMA-SPM suspensions were purified by centrifugation at 5100 × *g* for 10 min, filtrated through a filter, refrigerated at −80 °C in an ultra-low temperature freezer (Sanyo, Osaka, Japan) for 30 min, and lyophilized (Eyela, Tokyo, Japan) in the presence of 4% (w/v) mannitol at −80 °C over 36 h. 0.1% (w/v) SQV was mixed with 0.6% (w/v) lyophilized PBCA or MMA-SPM in DPBS. SQV was adsorbed onto PBCA and MMA-SPM in a baths-reciprocal shaker at 150 rpm and 37 °C for 3 h. The SQV-loaded polymers were stabilized by polysorbate 80 at 150 rpm and 37 °C for 30 min. The polysorbate 80-stabilized PBCA and MMA-SPM were treated by EMF with continuous electromagnetic wave of 5 mW and 915 MHz for 90 min. After ultracentrifugation (5415D, Eppendorf AG, Hamburg, Germany) at 11,500 × *g* for 1 h, loading efficiency was evaluated by high performance liquid chromatography (HPLC, Jasco, Tokyo, Japan) with a UV–vis spectrophotometer (UV-2075 Plus, Jasco, Tokyo, Japan) at 239 nm. Two high pressure pumps (PU-2080 Plus, Jasco, Tokyo, Japan) in series were applied to the mobile phase containing gradient of acetonitrile from 5 to 45% with a flow rate of 0.85 ml/min for 20 min. On the other hand, SQV-entrapped SLN was prepared by the method described previously (Kuo and Su, 2007) with minor modifications. Concisely, 7% (w/v) lipid, containing CB and docosanoic acid, SQV, 7% (w/v) PC, 3% (w/v) cholesteryl hemisuccinate, and 10% (w/v) taurocholate were mixed at 85 °C. The liquid was added drop by drop into ultrapure water at 3 °C under magnetic stirring for 15 min with lipid-to-water ratio of 1:10. SLN suspension was treated with continuous electromagnetic wave of 5 mW and 915 MHz for 90 min, filtrated and centrifuged at 14,000 × *g* for 30 min. Molecular SQV in supernatant was analyzed by HPLC followed by UV, and SLN pellet was resuspended with 2% (w/v) D-trehalose, refrigerated at −80 °C for 30 min and lyophilized. Entrapment efficiency (EE) of SQV was defined by EE = (total weight of SQV − weight of molecular SQV in supernatant)/total weight of SQV. For fluorescent carriers, FITC-conjugated dextrain were incorporated as stabilizer, loading agent, or entrapping substance.

Particle size distribution of SQV-incorporated carriers was obtained by a zetasizer 3000 HS_A with photo-correlation spectroscopy (Malvern, Worcs, UK). Infrared absorption spectra were analyzed by a Fourier-transform infrared spectrometer (FTIR, Shimadzu, Columbia, MD). Particle sizes of PBCA, MMA-SPM, and SLN were controlled, respectively, by reaction period, APS concentration, and stirring rate. The average diameters of PBCA, MMA-SPM, and SLN were, respectively,