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Enhanced brain distribution and pharmacodynamics of rivastigmine by liposomes following intranasal administration

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ABSTRACT

Alzheimer's disease (AD) is a common progressive neurodegenerative disorder associated with cholinergic neurons degeneration. The blood–brain barrier (BBB) not only provides protection for the brain but also hinders the treatment and diagnosis of this neurological disease, because the drugs must cross BBB to reach the lesions. The present work was aimed at formulating rivastigmine liposomes (Lp) and cell-penetrating peptide (CPP) modified liposomes (CPP-Lp) to improve rivastigmine distribution in brain and proceed to enhance pharmacodynamics by intranasal (IN) administration and minimize side effects. The results revealed that Lp especially the CPP-Lp can enhance the permeability across the BBB by murine brain microvascular endothelial cells model in vitro. IN administration of rivastigmine solution and rivastigmine liposomes demonstrated the capacity to improve rivastigmine distribution and adequate retention in CNS regions especially in hippocampus and cortex, which were the regions most affected by AD, than that of IV administration. Importantly, the lagging but intense inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities were relative to the extended release, absorption and retention. In addition, there was very mild nasal toxicity of liposomal formulations. The data suggest that rivastigmine liposomes especially CPP-Lp improve the brain delivery and enhance pharmacodynamics which respect to BBB penetration and nasal olfactory pathway into brain after IN administration, and simultaneously decrease the hepatic first pass metabolism and gastrointestinal adverse effects.

1. Introduction

Alzheimer's disease (AD), the most common neurodegenerative disease, is characterized by synaptic loss and degeneration of cholinergic neurons in the cortex and other areas of the brain, which are resulting deficits in cholinergic transmission and acetylcholine (ACh) level (Pakaski and Kalman, 2008). Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) can hydrolyze ACh in the brain (Mutlu and Degim, 2005). Cholinesterase inhibitors (ChEIs) catalyze the breakdown of AChE in synaptic cleft, thus enhancing ACh level to moderate AD. Rivastigmine is a reversible, non-competitive and carbamate-type dual ChEIs of brain AChE and BuChE simultaneously (Spencer and Noble, 1998), which is widely used as the symptomatic treatment of AD with mild-to-moderate dementia.

Rivastigmine is presently on the market delivered orally in the form of tablets and capsules. Unfortunately, there are limitations of oral therapy of rivastigmine including hepatic first pass metabolism and clearance, gastrointestinal destruction of the drug by digestive enzymes and acidic pH conditions of the digestive tract, inferior and unpredictable uptake and bioavailability, and gastrointestinal adverse effects, in severe cases, irreparable esophageal tears (Tenovuo, 2005; Venkatesh et al., 2007). More seriously, the blood–brain barrier (BBB) provides protection for the brain but hinders the treatment and diagnosis of neurological diseases because the drugs must cross the BBB to reach the lesions. Systemic drug delivery by nasal route is currently receiving considerable attention because this route has shown a noninvasive and acceptable administration of various drugs, avoidance of the hepatic first pass metabolism and the preferential drug delivery to brain via olfactory pathway while bypassing the BBB (Illum, 2003). This alternate route from nasal mucosa to brain has been achieved faster and higher drug absorption and has been used feasibly with tacrine

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(Jogani et al., 2008), nimodipine (Zhang et al., 2004), diazepam (Kaur and Kim, 2008) in the treatment of central nervous system (CNS) diseases and so on. In our previous study, the distribution of rivastigmine and its relation to pharmacodynamic effects, the inhibition of AChE and BuChE following intranasal (IN) and intravenous (IV) administration in rats were investigated (Yang et al., 2012). It has been shown that IN administration of rivastigmine had the capacity to improve distribution and pharmacological effect of rivastigmine in CNS regions compared to IV administration and the IN route can be an advantageous strategy for delivering rivastigmine into brain.

Like other CNS disease, the treatment of AD is particularly challenging because the therapeutic molecules must be transported not only across the brain cell membrane but also across the BBB. Nanoparticles have promising applications for drug delivery as well as for the diagnosis and treatment of several pathologies, such as those related to the CNS. Cell-penetrating peptides (CPPs) are a collection of different families of short peptides believed to enter cells by penetrating cell membranes (Trabulo et al., 2010; Zhang et al., 2009). CPPs have been widely exploited for the intracellular delivery of various cargoes such as proteins, siRNA and nanocarrier systems including liposomes and nanoparticles. Although the explicit mechanism of internalization of CPPs is unclear, there appears to be two kinds of mechanisms, transduction pathway and endocytosis pathway (Ma et al., 2011; Torchilin, 2008).

In order to catch on whether liposomes and CPP improve brain distribution of rivastigmine, minimize peroral side effects by IN routes, the rivastigmine liposomes (Lp) and CPP-modified liposomes (CPP-Lp) were prepared. Transport efficiency cross BBB of liposomal rivastigmine was comparatively evaluated with rivastigmine solution in murine brain microvascular endothelial cells (BMVECs) model. Distributions in CNS regions, plasma, and peripheral tissues as well as pharmacodynamic effects of these formulations were compared by determination of rivastigmine concentration, and AChE and BuChE activity in rats. Finally, toxicities of liposomal formulations were observed by mucosa lesion, ciliary movement and hemolysis evaluation.

2. Materials and methods

2.1. Materials and animals

Rivastigmine was obtained from Beijing Intermediate Imp. & Exp. Co., Ltd. (Beijing, China). 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino (polyethylene glycol)$_{2000}$] (DSPE-PEG-NHS) was purchased from NOF Corporation (Tokyo, Japan). Egg phosphatidylcholine (EPC) was got from Sigma–Aldrich (St. Louis, MO, USA). Cholesterol (Chol) was from Wako Pure Chemical Industries, Ltd. (Odaka, Japan). Sephadex G-50 was obtained from Pharmacia Biotech (Piscataway, NJ, USA). The CPP peptide (Gly-Leu-Pro-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg) was synthesized by GL Biochem Ltd. (Shanghai, China). AChE and BuChE Assay Kit were purchased from Nanjing Jiancheng Bioengineering Institution (Nanjing, China). Antipyrine was purchased from Beijing Huamaike Biotechnology Co., Ltd. (Beijing, China). All other chemicals used were of analytical or HPLC grade.

Murine brain microvascular endothelial cells (BMVECs, China–Japan Friendship Hospital, Beijing, China) were maintained in the endothelial cell culture medium (DMEM, 20% fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mmol/L L-glutamine, 100 μg/mL endothelial cell growth factor (ECGF) and 40 U/mL heparin).

Male Sprague-Dawley (SD) rats weighing 200 ± 30 g were obtained from Peking University Health Science Center (license No. SCXK (Jing) 2006–0008) and were housed under standard conditions with free access to food and water. The animals were fasted for at least 12 h prior to the experiment and were given water freely. Toads were obtained from Fangyuanyuan farm (Beijing, China). All of the animal experiments adhered to the principles of care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of Peking University.

2.2. Synthesis of DSPE-PEG-CPP

CPPs were reacted with DSPE-PEG-NHS (1:1.25, mol/mol) in dimethyl formamide adding minute quantity of triethylamine under stirring for 24 h at ambient temperature. In the reaction, the terminal amino of peptide was covalently attached to the functional NHS of the lipid-PEG derivative. The reacted mixture were further put into a dialysis bag with cutoff molecular weight of 3500 Da and dialyzed against deionized water over 24 h in order to remove unreacted materials. The final solution was lyophilized to dry powder. The molecular weight (MW) of the resulting product was determined by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS).

2.3. Liposomes preparation

Preparation of rivastigmine liposomes was carried out using ammonium sulfate gradient loading method. Firstly, two kinds of blank liposomes were prepared as following procedures: EPC, Chol (1:1, mol ratio) and EPC, Chol, DSPE-PEG-CPP (1:1:0.06, mol ratio) were dissolved with chloroform and methanol (4:1, v/v) in a pear-shaped flask and were subsequently evaporated to dry film using a rotary evaporator under vacuum. The lipid film was rehydrated and sonicated with 250 mM ammonium sulfate solution. The suspensions after hydration were successively extruded through polycarbonate membranes with the pore size of 400 nm, and 200 nm for 3 times, respectively. After extrusion, the suspensions were further dialyzed by the phosphate buffered saline (PBS, 50 mM KH_2PO_4, 15.2 mM NaOH, pH 6.5) over 6 h to obtain the blank liposomes. Secondly, rivastigmine was loaded into the blank liposomes. Namely, 5 mg of rivastigmine was added into 1 mL blank liposomes suspensions (50 mg lipid/mL) and incubated at 50 °C with intermittently shaken for 20 min to obtain the rivastigmine liposomes (Lp) and CPP-modified rivastigmine liposomes (CPP-Lp), respectively.

2.4. Liposomes characterization and drug release in vitro

2.4.1. Determination of rivastigmine concentration in vitro by HPLC

The rivastigmine concentration was determined by high performance liquid chromatography (HPLC) method with UV detector (Waters, USA). The mobile phase was consisted of acetonitrile: water (20 mM $NaH_2PO_4{\cdot}2H_2O$, 10 mM $Na_2HPO_4{\cdot}12H_2O$) (25: 75, v/v) at 1.0 mL/min of flow rate and 218 nm of wavelength using ODS column (Bonchrom, 250 mm × 4.6 mm).

2.4.2. Liposomes characterizations

The size, zeta potential and polydispersity index (PDI) of two liposomes were determined by dynamic light scattering (DLS) using Malvern Zetasizer 3000HSA (Malvern Instruments Ltd., UK).

The total content of rivastigmine (W_{total}) in liposomes suspension was determined after destroyed the liposomes by adding methanol. The rivastigmine encapsulated in liposomes ($W_{encapsulate}$) was determined after separated encapsulating rivastigmine from free one by gel filtration through a Sephadex G-50 column and eluted with pH 6.5 PBS. The encapsulation