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Rosmarinic acid- and curcumin-loaded polyacrylamide-cardiolipin-poly (lactide-*co*-glycolide) nanoparticles with conjugated 83-14 monoclonal antibody to protect β-amyloid-insulted neurons

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ABSTRACT

Polymeric nanoparticles (NPs) combined with lipids can have profound effects on treatment efficacy in patients with neurological disorders such as Alzheimer's disease (AD). We developed polyacrylamide (PAAM)-cardiolipin (CL)-poly(lactide-*co*-glycolide) (PLGA) NPs grafted with surface 83-14 monoclonal antibody (MAb) to carry rosmarinic acid (RA) and curcumin (CUR). This drug delivery system was used to cross the blood–brain barrier (BBB) and enhance the viability of SK-N-MC cells insulted with β-amyloid (Aβ) deposits. Experimental evidence revealed that an increase in the concentration of 83-14 MAb enhanced the permeability coefficient of RA and CUR using the nanocarriers. The levels of phosphorylated p38 and phosphorylated tau protein at serine 202 in degenerated SK-N-MC cells were in the order: Aβ > (Aβ + RA-CUR) > (Aβ + 83-14 MAb-RA-CUR-PAAM-PLGA NPs) > (Aβ + 83-14 MAb-RA-CUR-PAAM-CL-PLGA NPs) ≈ control. The viability of SK-N-MC cells reduced with time and CL in 83-14 MAb-RA-CUR-PAAM-CL-PLGA NPs advantaged Aβ-targeted delivery of RA-CUR. These results evidenced that the current 83-14 MAb-RA-CUR-PAAM-CL-PLGA NPs can be a promising pharmacotherapy to permeate the BBB and reduce the fibrillar Aβ-induced neurotoxicity.

1. Introduction

Alzheimer's disease (AD), a widespread irreversible dementia, is one of extremely troublesome neuropathological diseases in the central nervous system (CNS) with the death of affected patients occurring on an average of around 9 years after definite diagnosis [1]. The major AD symptoms include episodic memory, experience confusion, disorientation as to time, place and who one is, chronic cognitive impairment, personality change, and intellectual debility [2]. In AD pathology, the anatomical features of brain tissue typically reveal neurofibrillary tangles in the hippocampus, arteriosclerotic degeneration and cerebral atrophy [3]. Moreover, two most cardinal components – anomalous β-amyloid (Aβ) deposits in extracellular senile plaques and intracellular hyperphosphorylated tau protein – have been identified biochemically from AD patients [4,5]. In order to understand neurodegenerative traits of AD, scientists and physicians should employ reliable, practicable models of cholinergic neurons for neuroanalytical study. After induction with Aβ, a culture of SK-N-MC cells, which have active α7 nicotinic acetylcholine receptors involved in specific endocytosis and recognition, could be suitable for AD research [6,7]. In AD therapy, a number of antioxidants have been used to exterminate reactive oxygen species and retard the degeneration. For example, hydrophilic rosmarinic acid (RA) has an ability to eliminate peroxynitrite anions and reduce inflammatory responses [8]. Hydrophobic curcumin (CUR) is also a competent antioxidative chemical in the prevention of free radical formation and did not induce apparent adverse reactions in AD patients [9]. Therefore, combination of RA and CUR may have great bioactivity to rescue apoptotic neurons from Aβ insult. However, fast degradation and limited bioavailability of RA and CUR in the CNS detract their medicinal applicability. To improve the quality of RA and CUR in physiology, conjugation of bioactive element in drug carrier system can be a feasible strategy to facilitate brain uptake and AD-targeted delivery of the two drugs.

Poly(lactide-*co*-glycolide) (PLGA), a biodegradable polymer, can be decomposed into lactic and glycolic acids and converted further into carbon dioxide through the tricarboxylic acid cycle [10]. PLGA could form uniform nanoparticles (NPs) in microemulsion [11]. A study on delivering pharmaceuticals against human immunodeficiency virus residing in the brain showed that antiretroviral nevirapine-entrapped PLGA NPs could permeate human brain-microvascular endothelial cells (HBMECs), the key cellular component of the blood–brain barrier (BBB) [12]. Also, polyacrylamide (PAAM) is a synthetic cationic biopolymer

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with good biocompatibility [13]. PAAM NPs could be useful as photosensitizer carriers to inhibit the growth of human Caucasian colon adenocarcinoma cells [14]. A photodynamic therapy study showed that PAAM NPs improved tumor uptake and revealed potential for long-term cancer cure and tumor imaging *in vivo* [15]. With regard to drug targeting, 83-14 monoclonal antibody (MAb) can regulate the penetration of substances into the brain via receptor-mediated endocytosis [16]. 83-14 MAb plays an important role in docking insulin receptors in HBMECs and targeting the CNS [17]. The strong binding of 83-14 MAb to HBMECs triggered transcytosis of solid lipid nanoparticles and improved the transport of antitumor etoposide to retard the proliferation of glioblastoma multiforme [18]. Thus, 83-14 MAb may be a functional biomolecule to deliver polymeric PAAM-PLGA composite across the BBB. In addition, cardiolipin (CL), a distinctive phospholipid comprising two anionic phosphorus groups and four hydrocarbon tails, is efficacious to bind to Aβ [19]. It is intriguing that soluble Aβ aggregates could have a strong interaction with CL component in mitochondrial membrane [20]. In fact, CL is involved in stabilization of membrane structure, activation of enzymes and mitochondrial apoptosis [21]. Hence, incorporation of CL in nanocarriers may benefit topical release of RA and CUR to Aβ-insulted cerebral tissue and mitigate degenerative progression in AD management.

The aim of this study was to investigate the capability of RA- and CUR-loaded PAAM-CL-PLGA NPs with grafted 83-14 MAb (83-14 MAb-RA-CUR-PAAM-CL-PLGA NPs) to inhibit apoptosis of Aβ-insulted neurons. Since poor efficacy of antioxidants in permeating the CNS and treating the symptoms of AD is often encountered, improved medication of RA and CUR with targeting to the BBB for neuronal recovery is inevitable. We analyzed the physicochemical properties of 83-14 MAb-RA-CUR-PAAM-CL-PLGA NPs, recognition function of the formulations, delivery of RA and CUR to the BBB, expression of phosphorylated mitogen-activated protein kinase (MAPK, phosphorylated p38 (p-p38)) and phosphorylated tau protein (phosphorylated tau protein at serine 202 (p-S202)), and rescue of degenerated SK-N-MC cells.

2. Materials and methods

2.1. Materials

Ethyl acetate, CUR, PAAM, RA, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), fluorescein isothiocyanate (FITC), QuantiPro bicinchoninic acid (BCA) protein assay kit, phosphotungstic acid (PTA), Dulbecco's phosphate buffered saline (DPBS), trypsin-ethylenediaminetetraacetic acid (trypsin-EDTA), gelatin, propidium iodide (PI), formalin, 4′,6-diamidino-2-phenylindole (DAPI), 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), human fibronectin, sodium dodecyl sulfate (SDS), and polyacrylamide gel (PAG) were purchased from Sigma-Aldrich (St. Louis, MO). 1′,3′-bis[1,2-dimyristoyl-*sn*-glycero-3-phospho]-*sn*-glycerol (CL) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[carboxy (polyethylene glycol)-2000] (DSPE-PEG(2000)-CA) were obtained from Avanti Polar Lipids (Birmingham, AL). Tetrahydrofuran (THF) and dimethyl sulfoxide (DMSO) were purchased from J.T. Baker (Phillipsburg, NJ). *N*-hydroxysuccinimide (NHS) and Triton-X-100 were obtained from Acros (Morris, NJ). 83-14 MAb and anti-insulin receptor antibody were purchased from Invitrogen (Carlsbad, CA). Tris hydroxymethyl aminomethane (Tris) and hydrochloric acid were obtained from Riedel-de Haen (Seelze, Germany). HBMECs were purchased from Biocompare (South San Francisco, CA). HAs, endothelial cell medium, and astrocyte medium were obtained from Sciencell (Corte Del Cedro Carlsbad, CA). SK-N-MC cells (from human neuroblastoma) were purchased from American Type Tissue Collection (Rockville, MD). Polyethylene terephthalate membrane (PET membrane) and transwell were obtained from BD Falcon (Franklin Lakes, NJ). $A\beta_{1-42}$ powder, minimum essential medium, fetal bovine serum, and sodium pyruvate solution were purchased from Life Technologies (Carlsbad, CA). Tris-buffered saline with Tween 20 (TBST), anti-p38 antibody (phospho Thr180&Tyr185) (anti-p-p38), anti-tau antibody (phospho serine 202) (anti-p-S202), and anti-glyceraldehyde 3-phosphate dehydrogenase antibody (anti-GAPDH) were obtained from Cell Signaling (Danvers, MA). Lysis buffer, bovine serum albumin standard, goat anti-rabbit immunoglobulin G (IgG; heavy and light (H&L)) with horseradish peroxidase (HRP) conjugate, and anti-Aβ monoclonal antibody were purchased from Abcam (Cambridge, MA). Polyvinylidene fluoride (PVDF), enhanced chemiluminescence (ECL), and select ECL were obtained from GE Healthcare (Buckinghamshire, England). PLGA (85:15, 58,000 g/mol) was purchased from Purac (Bingen, Germany), ultrapure water from Barnstead (Dubuque, IA), genipin from Challenge Bioproducts (Taichung, Taiwan), membrane tube (12.4 kDa) from Spectrum Laboratories (Rancho Dominguez, CA), methanol from Mallinckrodt Baker (Phillipsburg, NJ), serum blocking solution from Zymed (South San Francisco, CA), 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) from Biological Industries (Beit-Haemek, Israel), polyclonal secondary antibody to mouse IgG H&L with rhodamine conjugate from Millipore (Billerica, MA), aqueous mounting medium from Bio SB (Santa Barbara, CA), penicillin-streptomycin-glutamate (PSG) solution from Gibco (Carlsbad, CA), Coomassie blue assay kit from Thermo Fisher Scientific (St. Waltham, MA), and transfer buffer from Bio-Rad (Hercules, CA).

2.2. Preparation of 83-14 MAb-RA-CUR-PAAM-CL-PLGA NPs

2.2.1. Preparation of RA-CUR-PAAM-CL-PLGA NPs

9–10 mg of PLGA, 0–1 mg of CL, and 0.5 mg of DSPE-PEG(2000)-CA were dissolved in 10 mL of ethyl acetate at 400 rpm for 1 h. The total weight of PLGA and CL in a single batch was fixed at 10 mg. 250 μg/mL of CUR were added to the above solution. Fluorescent CL-PLGA NPs were fabricated by addition of 50 ppm of FITC to the mixture of PLGA, CL, and DSPE-PEG(2000)-CA. The hydrophobic phase was emulsified with 50 mL of surfactant solution containing 0.03% (w/v) Tween 80 (Fisher Scientific, Fair Lawn, NJ) in ultrapure water at 22000 rpm and 25 °C for 10 min. 140 mL of ultrapure water were added to the emulsion at 800 rpm for 1 h. The emulsion was added to a membrane tube and dialyzed against 200 mL of ultrapure water at 150 rpm and pH 8 for 1 h. CUR-CL-PLGA NPs were produced by the emulsification-diffusion-dialysis method. Newly formed CUR-CL-PLGA NPs were filtered and centrifuged (AVANTij-25, Beckman Coulter, Palo Alto, CA) at 159,000 × *g* for 10 min. 0.2–0.4 mg/mL of PAAM and 25 μg/mL of RA were dissolved in ultrapure water, mixed at 200 rpm for 10 min, and reacted with 0.01% (w/v) genipin at 200 rpm for 10 min. To prepare RA-CUR-PAAM-CL-PLGA NPs, 0.1% (w/v) CUR-CL-PLGA NPs was activated with 0.08% (w/v) EDC and 0.04% (w/v) NHS by stirring at 120 rpm for 2 h, conjugated with 0.05% (w/v) RA-PAAM solution at 120 rpm for 3 h, and centrifuged at 159,000 × *g* for 10 min. The bottom pellet was dried, frozen (Panasonic Healthcare, Gunma, Japan) at −80 °C for 30 min, and freeze dried (Eyela, Tokyo, Japan) at 2–4 Torr and −80 °C for 24 h.

2.2.2. Determination of unloaded RA and CUR from RA-CUR-PAAM-CL-PLGA NPs

To evaluate the quantity of RA, the supernatant was separated using a high performance liquid chromatograph (HPLC; Jasco, Tokyo, Japan) with a reverse phase BDS Hypersil C-18 column (Thermo Hypersil-Keystone, Bellefonte, PA). The mobile phase was composed of 1:1 (v/v) methanol in ultrapure water, was impelled using a high-pressure pump (PU-2080 Plus, Jasco) at a flow rate of 1 mL/min and analyzed using an ultraviolet (UV)–visible detector (UV-2075 Plus, Jasco) at 320 nm. In addition, to quantify CUR, RA-CUR-PAAM-CL-PLGA NPs were treated with methanol at 37 °C for 1 h, analyzed using an HPLC with a C-18 column heated (Alltech, Derrfield, IL) at 37 °C, and detected at 430 nm. The mobile phase was ultrapure water adjusted with citric acid to pH 3 containing THF gradient of 5–40% (v/v) for 20 min and was driven using two high-pressure pumps in series at a rate of 1 mL/min. The loading efficiency of RA in RA-CUR-PAAM-CL-PLGA NPs, E_{RA} (%), was