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UEA I-bearing nanoparticles for brain delivery following intranasal administration

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Abstract

Surface engineering of nanoparticles with lectins opened a novel pathway to improve the brain uptake of agents loaded by biodegradable PEG-PLA nanoparticles following intranasal administration. Ulex europeus agglutinin I (UEA I), specifically binding to L-fucose, which is largely located in the olfactory epithelium, was selected as a promising targeting ligand and conjugated onto the PEG-PLA nanoparticles surface with an optimized protocol relying on maleimide-mediated covalent binding technique. The *in vivo* results in rats suggested that UEA I modification at the nanoparticles surface facilitated the absorption of a fluorescent marker—6-coumarin associated with the nanoparticles into the brain following intranasal administration with significant increase in the area under the concentration–time curve (about 1.7 times) in different brain tissues compared with that of coumarin incorporated in the unmodified ones. UEA I-conjugation also elevated the brain-targeting efficiency of nanoparticles. Inhibition experiment of specific sugar suggested that the interactions between the nasal mucosa and the lectinised nanoparticles were due to the immobilization of carbohydrate-binding pockets on the surface of the nanoparticles. Distribution profiles of UEA I-modified nanoparticles indicated their higher affinity to the olfactory mucosa than to the respiratory one. Therefore, the UEA I-modified nanoparticles might serve as potential carriers for brain drug delivery, especially for mental therapeutics with multiple biological effects.

Keywords: Brain targeting; Nanoparticles; Ulex europeus agglutinin I; Intranasal administration

1. Introduction

Drug delivery to the brain is made difficult by the presence of the blood-brain barrier (BBB), which is formed by tight junctions within the capillary endothelium of the vertebrate brain (Pardridge, 1991). A great deal of efforts, therefore, have been made in developing ways to open, defeat or circumvent the BBB in order to deliver drugs from blood to brain. Intranasal administration offers a non-invasive alternative route to the central nervous system (CNS) for drug delivery, effectively bypassing the BBB (Graff and Pollack, 2005). Indeed, the past few years have witnessed a sharp increase in the amount of research on the nasal pathway for the CNS drug delivery (Graff and Pollack, 2005). However, the total amount of drugs accessing the brain were reported to be low, especially for nasally applied biotech drugs such as peptides, proteins and DNA, which were poorly absorbed and highly susceptible to the harmful environment of the nasal cavity (Chen et al., 1998; Kim et al., 2000; Dufes et al., 2003). The incorporation of these drugs into nanoparticles might be a promising approach, since colloidal formulations have been shown to protect them from the degrading milieu in the nasal cavity and facilitate their transport across the mucosal barriers (Vila et al., 2002). Besides, the resident time of nanoparticles in nasal cavity is limited because of mucociliary clearance (e.g. particle clearance within the nose every 15–20 min), which is not available for the complete absorption of the formulation (Vyas et al., 2005). This made bioadhesive formulations with enhanced permeability a better alternative.

The bioadhesive delivery system can be obtained by means of surface modification of drug carriers with biological ligands, which recognize and adhere to specific chemical structures on the surface of cells, thus facilitating absorption of the delivery

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system. A good example of this is that lectins, proteins or glycoproteins of nonimmunological origin, which specifically recognize sugar molecules and bind the glycosylated membrane components, were widely used to conjugate with colloidal carrier systems, such as liposomes or nanoparticles to improve oral drug absorption (Bies et al., 2004). Our recent work suggested that wheat germ agglutinin (WGA) modification on the surface of poly(ethylene glycol)-poly(lactic acid) (PEG-PLA) nanoparticles facilitated the uptake of fluorescence tracer embedded in the nanoparticles in the CNS following intranasal administration (Gao et al., 2006). Ulex europeus agglutinin I (UEA I), specifically binding to L-fucose, which is largely located in the olfactory epithelium (Lundh et al., 1989), was widely used as a neuronal marker (Gheri et al., 1991; Pellier and Astic, 1994). Previous research also suggested that UEA I-bearing liposomes exhibited effective targeting to Peyer's patch (Chen et al., 1996). Thus, the aim of this contribution was to present a protocol for surface engineering of PEG-PLA nanoparticles with UEA I and to evaluate its ability to mediate drug delivery to the brain following intranasal administration. To achieve this goal, maleimide-PEG-PLA was blended with PEG-PLA to prepare nanoparticles by simple emulsion/solvent evaporation. The resulting nanoparticles were then functionalized with thiolated WGA by taking advantage of the thiol group coupling activity of maleimide. In the study of the blood and brain uptake of the functionized nanoparticles, a lipophilic fluorescent probe with high sensitivity, 6-coumarin, which was widely used in the *in vitro* and *in vivo* experiments (Desai et al., 1997; Panyam and Labhasetwar, 2003; Lu et al., 2005), was incorporated into the nanoparticles, and the blood and brain tissue concentrations of the fluorescent marker associated to UEA I-conjugated nanoparticles were detected with a high performance liquid chromatography (HPLC)-fluorescence detection method.

2. Materials and methods

2.1. Materials and animals

The copolymers of methoxy-PEG-PLA (MePEG-PLA) and maleimide-PEG-PLA were synthesized by the ring opening polymerization of D,L-lactide (99.5% pure, PURAC) initiated by MePEG (M_W 3000 Da, SUNBRIGHT^{TM} MEH-30H, NOF Corporation, Lot no. 14530, Japan) and maleimide-PEG (M_W 3400 Da, NEKTAR^{TM}, Lot no. PT-08D-16, Huntsville, AL, USA), respectively, using stannous octoate as catalyst (Y. Zhang et al., 2004; Gao et al., 2006). UEA I and L-fucose were obtained from Sigma (USA), 6-coumarin from Aldrich (USA), 2-iminothiolane hydrochloride (2-IT) from Sigma (USA) and 5, 5-dithiobis (2-nitrobenzoic acid) (Ellman's reagent) from Acros (Belgium). Rabbit anti-nerve-specific enolase polyclonal antibody and Cy3-conjugated goat-anti-rabbit IgG were purchased from Wuhan Boster Biological Technology Ltd. Double-distilled water was prepared using a Millipore Simplicity System (Millipore, Bedford, USA). All the other materials were of analytical reagent grades and used without further purification.

Sprague–Dawley rats (180–220 g) were obtained from the Experimental Animal Center of Fudan University and maintained at 22 ± 2 °C on a 12 h light–dark cycle with access to food and water *ad libitum*. And animal experiments were carried out in compliance with the protocol of Animal Use and Care by the Medical Center of Fudan University.

2.2. Preparation of UEA I-conjugated PEG-PLA nanoparticles (UEA-NP)

2.2.1. Preparation of 6-coumarin-loaded PEG-PLA nanoparticles

PEG-PLA nanoparticles (NP) were prepared with a blend of maleimide-PEG-PLA and MePEG-PLA using the emulsion/solvent evaporation technique described by Tobio et al. (1998). Briefly, 50 μl of water was emulsified by sonication (220 W, 30 s) on ice using a probe sonicator (Scientz Biotechnology Co. Ltd., China) in 1 ml of a solution of 25 mg/ml of blend of maleimide-PEG-PLA and MePEG-PLA at different ratios in dichloromethane containing 2 mg/ml of 6-coumarin. This primary emulsion was then emulsified by sonication (220 W, 30 s) on ice in a 2 ml of 1% aqueous sodium cholate solution. And the w/o/w emulsion obtained was further diluted into 25 ml of a 0.5% aqueous sodium cholate solution under moderate magnetic stirring. Five minutes later, dichloromethane was evaporated at low pressure at 30 °C using Büchi rotavapor R-200 (Büchi, Germany). Then the nanoparticles were centrifuged at 21,000 × *g* for 45 min using TJ-25 centrifuge (Beckman Counter, USA) equipped with A-14 rotor. The supernatant discarded, and the obtained nanoparticles were subjected to a 1.5 cm × 20 cm sepharose CL-4B column and eluted with 0.05 M HEPES buffer (pH 7.0) containing 0.15 M NaCl to remove the 6-coumarin unentrapped or adsorbed to the exterior of the nanoparticles.

2.2.2. Surface modification of the nanoparticles

UEA I was radio labeled with iodogen methods as described previously (Sobal et al., 2004), purified, mixed with unlabeled lectin and thiolated for 60 min with a predetermined molar excess of 2-iminothiolane in 0.15 M sodium borate buffer, pH 8 supplemented with 0.1 mM EDTA. The product was then purified with Hitrap^{TM} Desalting column (Amersham Pharmacia Biotech AB, Sweden) with the protein fractions collected and introduced thiol groups determined spectrophotometrically (λ = 412 nm) with Ellman's reagent (Ellman, 1959).

Then the thiolated protein, contained a trace amount of ^{125}I-labeled UEA I, was incubated with different amounts of nanoparticles to react at room temperature for 4–16 h. The unconjugated UEA I was separated from nanoparticles by Sepharose CL-4B column chromatography.

2.3. Morphology and particle size

The morphological examination of nanoparticles was performed by transmission electron microscopy (TEM) (H-600, Hitachi, Japan) following negative staining with sodium phosphotungstate solution. The mean diameter of the nanoparticles was determined by dynamic light scattering (DLS) analysis