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Research paper

A novel small Odorranalectin-bearing cubosomes: Preparation, brain delivery and pharmacodynamic study on amyloid-β_{25-35}-treated rats following intranasal administration

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ABSTRACT

Because of the immunogenicity and toxicity *in vivo* of large molecules such as lectins, the application of these molecules is remarkably restricted in drug delivery systems. In this study, to improve the brain drug delivery and reduce the immunogenicity of traditional lectin modified delivery system, Odorranalectin (OL, 1700 Da), a novel non-immunogenic small peptide, was selected to establish an OL-modified cubosomes (Cubs) system. The streptavidin (SA)-conjugated Cubs were prepared by incorporating maleimide–PEG–oleate and taking advantage of its thiol group binding reactivity to conjugate with 2-iminothiolane thiolated SA; mono-biotinylated OL was then coupled with the SA-modified Cubs. The OL-decorated Cubs (OL-Cubs) devised *via* a non-covalent SA-biotin "bridge" made it easy to conjugate OL and determine the number of ligands on the surface of the Cubs using sensitive chemiluminescent detection. Retention of the bio-recognitive activity of OL after covalent coupling was verified by hemagglutination testing. Nose-to-brain delivery characteristic of OL-Cubs was investigated by *in vivo* fluorescent biodistribution using coumarin-6 as a marker. The relative uptake of coumarin carried by OL-Cubs was 1.66- to 3.46-fold in brain tissues compared to that incorporated in the Cubs. Besides, Gly14-Humanin (S14G-HN) as a model peptide drug was loaded into cubosomes and evaluated for its pharmacodynamics on Alzheimer's disease (AD) rats following intranasal administration by Morris water maze test and acetylcholinesterase activity determination. The results suggested that OL functionalization enhanced the therapeutic effects of S14G-HN-loaded cubosomes on AD. Thus, OL-Cubs might offer a novel effective and noninvasive system for brain drug delivery, especially for peptides and proteins.

1. Introduction

Being influenced by the frantic rhythm of life, population aging, environmental factor, traffic accidents and so on, brain diseases are becoming more and more serious threaten of human health with yearly progressive increase. Fortunately, the development of neuroscience has facilitated the discoveries of proteins [1] and peptides [2] with considerable potential in the treatment of central nervous system (CNS) diseases such as Alzheimer's disease (AD) and Parkinson disease. Thus, there is an increasing need of novel brain drug carriers for macromolecular drugs in the treatment of CNS disorders.

Direct nose-to-brain delivery of therapeutics, bypassing the blood–brain barrier (BBB), has provided a noninvasive and effective route for the treatment of CNS disorders. Numerous successful studies focusing on the nasal pathway for CNS drug delivery have been reported [3,4]. However, the uptake of drugs in the brain is reported to be low, especially nasally applied macromolecular drugs such as peptides, proteins and DNA, which are poorly absorbed and highly susceptible to the harmful environment of the nasal cavity. Incorporation of these drugs into polymeric nanoparticles, polymersomes [5] or liposomes might be a promising approach, since colloidal carrier systems have been shown to protect compounds from the degrading milieu in the nasal cavity and facilitate their transport across the mucosal barriers [6]. However, these nanocarriers still bear the problems of low drug encapsulation efficiency and payload, residual solvent and non-bioadhesive [7]. In recent years, by virtue of a variegated range of encapsulation, high drug payloads and stabilization of peptides

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[8,9] or proteins [10–13], cubic phase structures self-assembled using amphiphilic materials when present in an aqueous environment have become the focus of drug delivery systems. Cubosomes (Cubs) are discrete, submicron, nanostructured particles of bicontinuous cubic liquid crystalline phase, which are able to incorporate large amounts of drugs of varying physicochemical properties and can be localized in body cavities, on the skin or on different mucosal surfaces [14]. Cubs consisting of amphiphilic lipoid materials, such as glyceryl monooleate (GMO), have a stiff, bioadhesive and gel-like appearance and are degradable and innoxious in the human body [15]. In spite of academic [16,17] and commercial interests, there are few reports of studies on nasally administered Cubs for drug brain delivery. Nevertheless, as with plain-nanovehicles in nasal application, on the one hand, mucociliary clearance limits the time available for drug absorption, and on the other hand, selective transport (for drug targeting to the brain) following mucosal absorption is difficult to realize. Therefore, modified nanovehicles with both mucoadhesive properties and enhanced specific delivery to the brain might eliminate the obstacles related to drug brain delivery *via* the nasal route and offer an ideal alternative.

One approach to improve the mucoadhesive ability of nanoparticles is surface modification with bio-recognitive ligands such as lectins, of which the potential to act as drug delivery adjuvants is increasing [18–21]. In spite of the interesting biological potential of lectin for drug targeting and delivery, a potential disadvantage of natural lectins is the large size of these molecules, which results in immunogenicity and toxicity. Smaller peptides that can mimic the function of lectins are promising candidates for drug targeting. Odorranalectin (OL) from the skin secretions of the amphibian *Odorrana grahami*, specifically binds to L-fucose [22], which was shown to have greater expression on olfactory mucosa than on respiratory mucosa [23,24], is the smallest lectin (MW 1.7 kDa) [25] and was selected as a model ligand to enhance the binding of cubosomes to nasal mucosa and improve drug brain uptake.

S14G-HN, a derivative of Humanin (HN) with substitution of glycine for serine 14, shown about 1000-fold neuroprotective activity than HN [26], is a novel 24-amino acid peptide and effective at low concentrations against memory impairment caused by AD-related insults [27,28]. As a very promising anti-AD drug, S14G-HN has a high specificity and low doses merits. However, the peptide is hard to cross the BBB and easy to be metabolized by deactivation under internal circumstances, intravenous administration is difficult to achieve an ideal efficacy, and it often needs intraventricular injection [29]. So far, only Colivelin [30] (a hybrid peptide of HN and neurotrophic factor) and the other HN derivatives [31] reported were intranasally administered against memory impairment of AD models. And there are little reports about S14G-HN preparations for intranasal administration in rats. The lack of effective carriers and a convenient administration are the main problems or common failing of these macromolecular drugs brain delivery. Therefore, S14G-HN was selected as a model drug in this study.

In brief, the present study attempted to develop a protocol for the surface engineering of poly (ethylene glycol) (PEG)-ylated cubosomes with OL (OL-Cubs) and to evaluate its brain delivery following intranasal administration. To do this, maleimide–PEG–oleate was synthesized and blended with 1-Monoolein and Pluronic F127 to prepare the cubosomes by dilution–sonication [32]. The resulting cubosomes were then functionalized with thiolated streptavidin (SA) by taking advantage of the thiol group coupling activity of maleimide. The mono-biotinylated OL (homing molecule) was then coupled to the cubosomes by a "bridge" of SA-biotin and a PEG compound linker (Fig. 1). Coumarin-6, a lipophilic fluorescent probe with high sensitivity, was incorporated into cubosomes to investigate the brain distribution of OL-Cubs *in vivo*. In

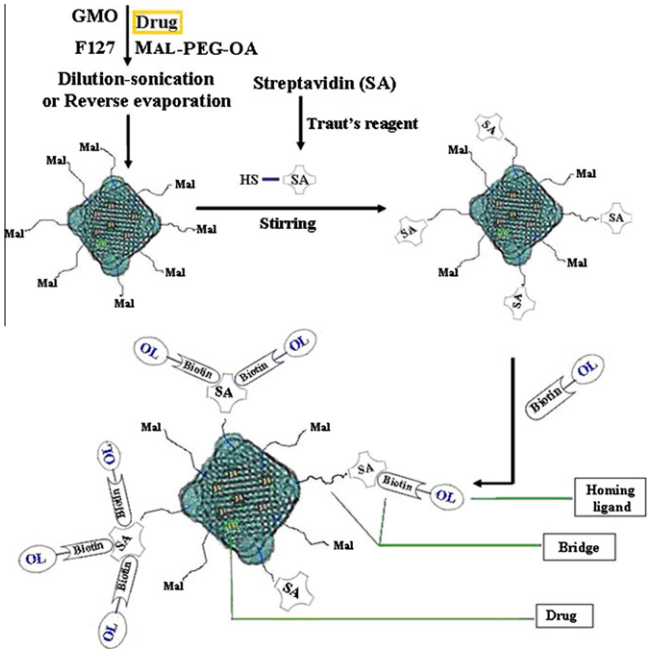

Fig. 1. Schematic illustration of preparing drug-loaded cubosome decorated by OL. The "sponge" structure self-assembled is composed of a common surfactant GMO and polymeric stabilizer Pluronic F127, with lipophilic (dilution–sonication method) or hydrophilic (reverse evaporation method) drugs encapsulation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the pharmacodynamic study, neuroprotective effect of the S14G-HN-loaded OL-Cubs was tested in $A\beta_{25-35}$-treated rats following intranasal administration.

2. Materials and methods

2.1. Materials and animals

1-Monoolein, denoted as RYLO™ MG pharma 19 glycerol monooleate (GMO), was a generous gift from Danisco (China) Co., Ltd. (Shanghai). PEO_{98}–PPO_{67}–PEO_{98} triblock copolymer (F127) was obtained from BASF Svenska AB (Helsingborg, Sweden). Maleimide poly (ethylene glycol) (MAL–PEG, MW 3500 Da, purity more than 90%) was supplied by Beijing JenKem Technology Co., Ltd. Oleoyl chloride, 6-coumarin, 2-iminothiolane hydrochloride (2-IT) and streptavidin (SA) were all purchased from Sigma–Aldrich (Shanghai) Trading Co., Ltd. Triethylamine (TEA) and dichloromethane were freshly dried according to conventional methods. Streptavidin-modified polystyrene beads (0.56 μm) were purchased from Bangs Laboratories; 5,5-dithiobis (2-nitrobenzoic acid) (Ellman's reagent) was from Acros (Belgium). The biotinylated Odorranalectin (biotin-YASPKCFRYPNGVLACT, biotin-OL, purity by HPLC >95%) and S14G-Humanin (purity by HPLC >95%) were both customized by GL Biochem (Shanghai) Ltd. Double-distilled water was purified by a Millipore Simplicity System (Millipore, Bedford, MA, USA). All other chemicals used were of analytical grade and without further purification.

Sprague-Dawley rats (180–220 g, ♀, License number: SCXK (Hu) 2008-0016) were obtained from the Experimental Animal Center of Fudan University and maintained at 22 ± 2 °C on a 12 h light-dark cycle with *ad libitum* access to food and water. Toads (30–40 g, ♂♀), also purchased from the Experimental Animal Center of Fudan University, were kept in moist conditions with free access to food and water. The animals used for the experiments were treated according to protocols evaluated and approved by the ethical committee