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Nose-to-brain delivery of BACE1 siRNA loaded in solid lipid nanoparticles for Alzheimer's therapy

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ABSTRACT

We designed a delivery system to obtain an efficient and optimal nose-to-brain transport of BACE1 siRNA, potentially useful in the treatment of Alzheimer's disease. We selected a cell-penetrating peptide, the short peptide derived from rabies virus glycoprotein known as RVG-9R, to increase the transcellular pathway in neuronal cells. The optimal molar ratio between RVG-9R and BACE1 siRNA was elucidated. The complex between the two was then encapsulated. We propose chitosan-coated and uncoated solid lipid nanoparticles (SLNs) as a nasal delivery system capable of exploiting both olfactory and trigeminal nerve pathways. The coating process had an effect on the zeta potential, obtaining positively-charged nanoparticles, and on siRNA protection. The positive charge of the coating formulation ensured mucoadhesiveness to the particles and also prolonged residence time in the nasal cavity. We studied the cellular transport of siRNA released from the SLNs using Caco-2 as a model of epithelial-like phenotypes. We found that siRNA permeates the monolayer to a greater extent when released from any of the studied formulations than from bare siRNA, and primarily from chitosan-coated SLNs.

1. Introduction

Direct nose-to-brain transport of drugs or biologics (proteins, oligonucleotides, or viral vectors) is feasible via the olfactory or trigeminal nerve system [1–5]. Olfactory nerve axons originating in the olfactory bulb (OB) penetrate the cribriform plate and terminate at the apical surface of the olfactory neuroepithelium (1–2 cm^2 surface area) [1,3,4]; it is located at the roof of the nasal cavity [6]. Moreover, filaments of the olfactory nerves are present both in the anterior and posterior parts at the middle turbinate. In addition, the respiratory mucosa (150 cm^2 surface area) is densely innervated by sensory and parasympathetic trigeminal nerves and is even more extensive than the olfactory nerve. Sensory maxillary branches innervate the deepest nasal segments, including the olfactory cleft [4]. Unlike olfactory sensory neurons, the trigeminal nerve endings do not penetrate the mucosal surface. Access of molecules to the dense network of trigeminal nerve endings is limited by their ability to cross the mucosal layer [2,4].

Transfer of substances into the brain occurs by slow intra-axonal transport or by faster transfer along the perineural space surrounding the nerve cells into the cerebrospinal fluid (CSF) and/or into the interstitial fluid of the brain [4]. In fact, some substances may be transported by an intracellular pathway within neurons following adsorptive, receptor-mediated, or non-specific fluid phase endocytosis. If they cross the olfactory epithelium, they can reach the OB by intraneuronal transport; alternatively, intracellular pathways across the respiratory epithelium potentially include endocytosis into peripheral trigeminal nerve processes resulting in intracellular transport to the brainstem [2]. Other substances may cross either the olfactory or respiratory epithelia by paracellular or transcellular transport, reaching the lamina propria and then the cranial compartment or into general circulation [2]. The presence of tight

Abbreviations: BACE1, β-secretase responsible for amyloid-β (Aβ) generation in the brain; BBB, blood–brain barrier; Caco-2, human epithelial colorectal adenocarcinoma cells; C, chitosan coated nanoparticles; CNS, central nervous system; CPPs, cell-penetrating peptides; CS, low molecular weight chitosan; CSF, cerebrospinal fluid; 6-FAM, 6-carboxyfluorescein; NR, uncoated nanoparticles; OB, olfactory bulb; PVA, poly(vinyl alcohol); RVG-9R, Chimeric Rabies Virus Glycoprotein fragment; SLN, solid lipid nanoparticles.

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junctions at the epithelial barrier as well as a mucus layer covering the respiratory and olfactory area of the nasal cavity limit paracellular permeability by molecules [3].

The ability to deliver therapeutically-relevant amounts of drugs directly from the nasal cavity to the central nervous system (CNS) to treat neurological diseases is dependent on the availability of efficient drug delivery systems [7]. Generally, nanoparticles increase the drug concentration of the encapsulated drug in the CSF or brain tissue after intranasal administration as compared to the same drug administered as a simple solution [7]. A penetration enhancer and a mucoadhesive polymer are often included in the formulation to increase drug permeability and decrease mucociliary clearance [5,8–14].

Recently, intranasal delivery has been proposed as a means to increase the delivery of small interfering RNA (siRNA) to the CNS [15–19]. These entities are potential therapeutic agents for many genetically-influenced diseases (Alzheimer's disease, Parkinson's disease and brain tumors) [20], but the clinical utilization has been limited because they have short half-lives as a result of poor *in vivo* stability and poor cell membrane permeance/permeability [21]. Moreover, because naked siRNA molecules are water-soluble and carry a net negative charge, they are subject to excretion from the mucosa following administration [17].

A variety of delivery strategies have been developed to overcome these limitations of the clinical utilization of siRNA: cell-penetrating peptides (CPPs) [22,23], cationic nanoemulsion of omega-3 fatty acids [24], methoxy poly(ethylene glycol)/polycaprolactone copolymers conjugated with a cell-penetrating peptide [17], and gelatin nanoparticles [19].

Of these, CPPs have already shown a great ability to improve therapeutic molecule delivery to treat CNS diseases [25]. Neurotoxins are often used as drug carriers to specifically target the CNS [26]. In particular, the 29-amino-acid peptide derived from the rabies virus glycoprotein (RVG) binds to acetylcholine receptors, which are highly expressed in the neuronal cells and the CNS. This peptide is able to conjugate siRNAs instead of using electrostatic complexation between the oligonucleotide and the 9 arginine residues of the RVG peptide (RVG-9R siRNA) [26]. Nicotinic acetylcholine receptor is expressed in the nasal cavity, presumably on intraepithelial trigeminal nerve endings [27] and in the OB [28].

The aim of this work was to propose a nasal delivery system capable of exploiting both olfactory and trigeminal nerve pathways to promote siRNA delivery/transport to the CNS. Specifically:

(1) the complex between RVG-9R and BACE1 siRNA was prepared to protect the oligonucleotide and enhance the intracellular pathway by receptor-mediated endocytosis within the neurons;
(2) the complexes in solid lipid nanoparticles (SLNs) were encapsulated to favor their transport across the olfactory epithelium and to increase penetration across the mucosal surface of the respiratory epithelium so that the trigeminal nerve endings could be reached;
(3) chitosan was used to modify nanoparticle surfaces to increase paracellular transport by opening the tight junctions and at the same time increase mucoadhesiveness to the system.

The major β-secretase responsible for amyloid-β (Aβ) generation in the brain is BACE1. BACE1 siRNA specifically influence the β-cleavage of amyloid precursor proteins and may be a potential therapeutic approach for treating Alzheimer's disease [29]. Lipid-based materials are the most widely-used biomaterials for nanoparticulate siRNA delivery [28].

2. Materials and methods

2.1. Materials

Fluorescence-labeled siRNA, targeting the BACE1 gene, was purchased from Sigma-Aldrich (Milan, Italy). The siRNA sequence was as follows: 5′-CUGUUAUCAUGGAGGGCUU-3′ (sense). The siRNA was labeled by 6-carboxyfluorescein (6-FAM) at the 3′ end of the sense strand (6-FAM–BACE1 siRNA). The chimeric RVG fragment (RVG-9R; sequence: YTIWMPENPRPGTPCDIFTNSRGKRASNGGGGRRRRRRRRR) was purchased from AnaSpec (Seraing, Belgium). Low-molecular-weight chitosan (CS), 82.6% deacetylated, viscosity 62 cP (1 wt% in 1% acetic acid at 25 °C), and polyvinyl alcohol (PVA), 87–90% hydrolyzed, MW 53,820, viscosity 6 cP (4% in H2O at 20 °C), were obtained from Sigma-Aldrich (Milan, Italy), as were DNA-free water and cell culture products. Witepsol E 85 solid triglycerides were kindle-provided by Cremer Oleo (Hamburg, Germany).

2.2. Electrophoretic mobility shift assay

6-FAM–BACE1 siRNA, 100 pmol, was incubated with the RVG-9R peptide at 1:0.1; 1:0.5; 1:1; 1:5, and 1:10 molar ratios (siRNA:peptide) for 15 min in dark conditions. The RNA-protein complexes were separated by electrophoresis using a 2% agarose gel in 0.5X TRIS-acetate/EDTA for 30 min at 80 V. Controls were siRNA sans peptide. Qualitative analysis of the oligonucleotide was evaluated through the fluorescence of the 6-FAM–BACE1 siRNA. To identify the peptide, the gel was colored with a Comassie solution (40% methanol, 10% acetic acid, and 0.25% Coomassie R Brilliant Blue; Sigma Aldrich, Milan, Italy) for 3 h under mild oscillation and then bleached (20% methanol and 10% acetic acid) overnight at 4 °C.

2.3. Preparation of nanoparticles

SLN were prepared using a modified solvent emulsification-evaporation method based on a w/o/w double-emulsion technique [30–32]. Uncoated nanoparticles (NR) as well as those coated with CS (C) were prepared. Briefly, 200 mg lipid was dissolved in 2 mL dichloromethane, then 0.2 mL RVG-9R/BACE1 siRNA complex solution at a 1:10 molecular ratio was added to the fatty mixture to form the primary emulsion. The emulsion was homogenized using a Bioblock Vibracell sonicator (Fisher Bioblock Scientific, Illkirch, France) for 30 s at 70% amplitude. This primary emulsion was poured into 10 mL PVA (2% w/v) and homogenized again under the same conditions. The resulting double emulsion was placed under magnetic stirring at room temperature to remove the organic solvent by evaporation.

A CS solution (1% w/v) was prepared by dissolving CS in water containing 1% (v/v) acetic acid (pH 4.6) and PVA (2% w/v). Nanoparticles were added at 1:1 w/w and the mixture was magnetically stirred overnight, allowing surface layer deposition of the polymer on the particles [33]. The same procedure was followed to prepare unloaded SLNs (NRb and Cb).

2.4. Nanoparticles characterization

After production, the nanoparticles were characterized for their average particle size (dm), polydispersity index (PI), and average zeta potential (ZP). All samples were diluted with Milli-Q water to a suitable concentration for both particle size and ZP analyses, which were performed using a 90Plus/90Plus/BI-MAS ZetaPlus (Brookhaven Instruments Corporation, NY, USA). All measurements were performed in triplicate.

Morphological characterization of SLNs was obtained using scanning electron microscopy. Samples were mounted onto metal