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Gelatin nanostructured lipid carriers-mediated intranasal delivery of basic fibroblast growth factor enhances functional recovery in hemiparkinsonian rats

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Abstract

Lipid nanoparticles with solid matrix have been given increasing attention due to their biodegradable status and ability to entrap a variety of biologically active compounds. In this study, new phospholipid-based gelatin nanoparticles encapsulating basic fibroblast growth factor (bFGF) were developed to target the brain via nasal administration. Treatment effects were assessed by quantifying rotational behavior, monoamine neurotransmitter levels and tyrosine hydroxylase expression in 6-hydroxydopamine induced hemiparkinsonian rats. The gelatin nanostructured lipid carriers (GNLs) were prepared by a water-in-water emulsion method and then freeze-dried. The GNLs possessed better profile than gelatin nanoparticles (GNs), with particle size 143 ± 1.14 nm and Zeta potential −38.2 ± 1.2 mV. The intranasal GNLs efficiently enriched exogenous bFGF in olfactory bulb and striatum without adverse impact on the integrity of nasal mucosa and showed obvious therapeutic effects on hemiparkinsonian rats. Thus, GNLs are attractive carriers for nose-to-brain drug delivery, especially for unstable macromolecular drugs such as bFGF.

From the Clinical Editor: This team of authors reports the development of phospholipid-based gelatin nanoparticles encapsulating basic fibroblast growth factor to target the brain via intranasal administration. A rat model of hemiparkinsonism was applied demonstrating a good safety profile and an obvious therapeutic effect.

Key words: Gelatin nanostructured lipid carriers; Nose-to-brain drug delivery; bFGF; Hemiparkinsonian

Intranasal delivery of large molecular weight biologics such as proteins, gene vectors, and stem cells is a noninvasive strategy to treat a variety of diseases/disorders of the central nervous system (CNS).[1] The major disadvantages of the route, aside from the challenge of reproducibility, are the limited absorption across the nasal epithelium and short resident time in nasal cavity that

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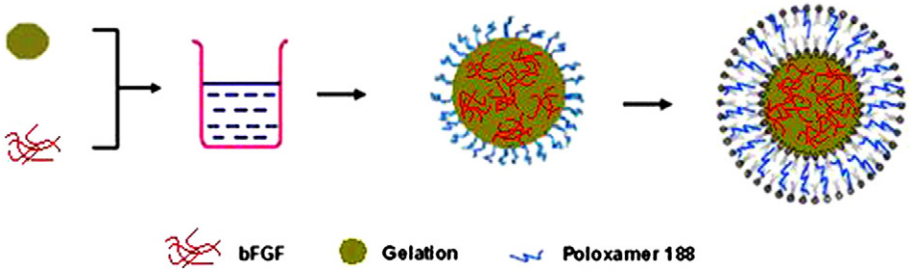

Figure 1. Schematic diagram of the bFGF-GNLs preparation. The bFGF-GNLs were prepared using water-in-water emulsion and freeze-drying technique.

restrict its application for particularly potent substances.[2,3] Vesicular systems have shown promising results in intranasal drug delivery of both small and large molecules to the CNS by overcoming limitations of nasal administration.[4] Intranasal mucoadhesive liposomes can enhance penetration of drug and provide better absorption into the brain compared to intranasal administration of drugs alone or oral administration.[5,6] However, drugs encapsulated in liposomes are not stable and are prone to leak. Nanoparticles may improve nose-to-brain drug delivery since they are able to protect the encapsulated drug from biological and/or chemical degradation, and promote extracellular transport by P-gp efflux proteins.[7] Recently, lipid nanoparticles with a solid matrix, including solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs), received major attention as novel colloidal drug carriers for intranasal delivery because they combine the advantages of polymeric nanoparticles, fat emulsions, and liposomes, and avoid some of their disadvantages.[8]

Parkinson's disease (PD) is a chronic CNS disorder caused primarily by the progressive loss of dopaminergic cells in the substantia nigra pars compacta (SNc). In PD patients, expressions of brain-derived neurotrophic factor (BDNF) and bFGF were reduced in the remaining dopaminergic neurons of SNc.[9-12] BDNF depletion from the midbrain-hindbrain selectively leads to reduced tyrosine hydroxylase (TH) expression.[13] In neurotoxin-induced degeneration models of midbrain DA neurons, higher levels of DA neuron death occurred in bFGF null mutant mice whereas more DA neurons were preserved in bFGF overexpressing mice,[14] suggesting that bFGF protects DA neurons from the neurotoxicity. Neurotrophic factor (NTF) therapy has recently gained attention in the treatment of PD.[15] The intact BDNF can be made to cross the BBB by a high-capacity, saturable transport system,[16] but bFGF is unstable in solution and has very short half-life[17]; thus, it does not cross the blood–brain barrier (BBB) in pharmacologically significant amounts,[18,19] which limits its therapeutic value in the CNS.

The aim of the present study was to determine whether gelatin nanostructured lipid carriers (GNLs) encapsulated bFGF (bFGF-GNLs) could be efficiently delivered to the brain striata with effective bioactivity via nasal epithelium. Physicochemical characterizations of GNLs and its bFGF preparation, including micromorphology, particle size, polydispersity index and Zeta potential, were investigated. The neuroprotective effect of bFGF-GNLs was evaluated in hemiparkinsonian rats following intranasal administration.

Methods

Materials and animals

All reagents and animals used in this study were commercially available. The animals were handled according to protocols approved by the ethical committee of Wenzhou Medical University and all the experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Supplemental Experimental Procedures).

Preparation of gelatin nanostructured lipid carriers

The schematic diagram of the preparation for bFGF-loaded nanoparticles is shown in Figure 1. The GNLs and its bFGF preparation were prepared using water-in-water emulsion and freeze-drying technique,[20-22] as described in Supplemental Experimental Procedures. The final bFGF concentrations in bFGF-GNs and bFGF-GNLs suspensions were 2 mg/mL, respectively.

Characterization of GNs and GNLs

The microscopic appearance of loaded or unloaded GNs and GNLs were examined by scanning electron microscopy (SEM). Zeta potential of the loaded or unloaded GNs and GNLs was determined by dynamic light scattering using a Zeta Potential/Particle Sizer Nicomp™ 380 ZLS (PSS. Nicomp, Santa Barbara, CA, USA). The encapsulating efficiency, loading capacity, and bioactivity of bFGF in GNs and GNLs were measured as previous reports.[23,24] The details were described in Supplemental Experimental Procedures.

6-OHDA induced hemiparkinsonian rat model

The hemiparkinsonian rats were generated by injecting 10.0 μl 6-OHDA solution in the right-side striatum (or vehicle for sham animals) by use of the stereotaxic apparatus,[25,26] as described in Supplemental Experimental Procedures.

Behavioral evaluation of apomorphine-induced rotations in hemiparkinsonian rats

The post-operative rats received a subcutaneous injection of DA agonist apomorphine (0.5 mg/kg), and were transferred into an opaque cylinder with 30 cm diameter which was placed 45 cm below the recording camera. After a 5 min habituation