Nose-to-Brain Delivery: Evaluation of Polymeric Nanoparticles on Olfactory Ensheathing Cells Uptake

TERESA MUSUMECI,[1] ROSALIA PELLITTERI,[2] MICHELA SPATUZZA,[2] GIOVANNI PUGLISI[1]

[1]Department of Drug Science, University of Catania, Catania 6-95125, Italy
[2]Institute of Neurological Sciences—CNR—Section of Catania, Catania 18-95126, Italy

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ABSTRACT: The nasal route has received a great deal of attention as a convenient and reliable method for the brain target on administration of drugs. When drugs are loaded into nanoparticles (NPs) the interaction with mucosa transports directly into the brain, skipping the blood–brain barrier and achieving rapid cerebrospinal fluid levels. Poly-lactic acid (PLA), poly-lactic-co-glycolic acid (PLGA), and chitosan (CS) were chosen to prepare NPs. After optimization of CS nanocarriers, our goal was to evaluate the different type of NPs uptake into olfactory ensheathing cells (OECs). We then correlated obtained biological data to zeta potential measurements of cells treated with NPs. Rodhamine-loaded NPs were used to study the uptake of OECs carried out by confocal microscopy at different times (1, 2, and 4 h). Our results showed that uptake of rodhamine-NPs by OECs was time dependent and it was influenced by the carrier charge. Confocal imaging of OECs demonstrated that NPPLGA showed a higher increase in uptake compared with NPPLA and NPCS after 1 h and it increased at 2–4 h. Zeta potential values of treated cells were more amplified with respect to untreated cells. The highest values were showed by unloaded NPPLGA, confirming microscopy data. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:628–635, 2014

Keywords: nasal drug delivery; nanoparticles; PLGA; cell culture; chitosan

INTRODUCTION

An area that is becoming more and more interesting in the pharmaceutical field is nasal drug administration, in particular nose-to-brain delivery. Both systemic and olfactory pathways for drug administration using this route were investigated. After nasal administration, drugs may be transported across the nasal membrane into the systemic circulation and, after crossing the blood–brain barrier (BBB), reach the brain (systemic pathway). The nasal pathway from the nose to the central nervous system (CNS) involves the direct transport of drugs into the brain tissues via the olfactory region of the nasal cavity (olfactory pathway).[1] In spite of several advantages (self-administration, low cost and increase compliance for patient, bypassing BBB), this route presented several limitations because of the site of administration (mucociliar clearance, drug degradation). In the past 10 years, nanotechnologies have waited to overcome these problems.[2] Polymeric nanoparticles (NPs) provide several advantages because of their controlled and prolonged effects on drug release and intracellular uptake. In fact, drug-loaded NPs increase drug absorption across membrane barriers.[3,4] In this decade, nose-to-brain delivery by drug delivery systems (DDSs) has taken the first steps and the most common investigated DDSs are polymeric NPs by chitosan (CS) and poly-lactic acid (PLA) and its derivatives. These materials are the most widely studied polymers, as a promising material, to obtain drug carriers. PLA and poly-lactic-co-glycolic acid (PLGA) are both approved by US Food and Drug Administration for pharmaceutical use.[4,5] They are biocompatible and biodegradable; also, they are widely used to prepare NPs for different routes of administration.[6,7] CS is a biodegradable polysaccharide: a copolymer of glucosamine and N-acetyl-D-glucosamine linked together by β-(1,4) glycosidic bonds. It has been used in pharmaceutical areas because it is biodegradable, biocompatible, and presents other interesting properties. Recent *in vivo* animal models studies demonstrated that NPs administrated intranasally reach CNS via the olfactory pathway and trigeminal nerve pathway.[8] The mammalian olfactory system is one of the few areas of the CNS that is capable of continuous neurogenesis throughout a lifetime.[9] When olfactory receptor neurons die, new neurons are produced by division of basal cells in the deepest layer of the epithelium.[10–13] This regenerative ability is due, in part, to particular glial cells of the olfactory nerve, olfactory ensheathing cells (OECs). They wrap up the olfactory nerve along its whole length, from the basal lamina of the epithelium to the olfactory bulb, crossing the peripheral nervous system–CNS junction. OECs share properties with both Schwann cells and astrocytes of CNS,[13,14] and they are a source of growth factors and adhesion molecules.[15–17] Consequently, in the last few years, OECs have drawn considerable interest because they are able to promote regeneration in the injured CNS.[18]

The aim of our work was to verify the possible uptake of the NPs into OECs to realize a drug carrier for intranasal administration. We evaluated three different common types of NPs used for nose-to-brain delivery. To achieve this goal, the first step of our work was characterized by the optimization of CS NPs, investigating the influence of different variables on mean size particles.

One CS nanosuspension (NPCS), NPPLA (nanoparticles obtained from PLA) and NPPLGA (nanoparticles obtained from

Abbreviations used: OECs, olfactory ensheathing cells; NPs, nanoparticles; PLA, poly-lactic acid; PLGA, poly-lactic-co-glycolic acid; CS, chitosan; PDI, polydispersity index; PCS, photon correlation spectroscopy; LMW, low molecular weight; MMW, medium molecular weight.

Correspondence to: Teresa Musumeci (Telephone: +39-095-738-4021; Fax: +39-095-738-4021; E-mail: teresa.musumeci@unict.it)

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PLGA)[6] was selected to load fluorescent marker (rhodamine), and a morphological and a physicochemical characterization were curried out on these formulations [photon correlation spectroscopy (PCS) and scanning electron microscopy (SEM)]. We evaluated the uptake process of the selected rhodamine-loaded NPs (NPPLA, NPPLGA, NPCS) on OECs by confocal microscopy at various lengths of time. Moreover, we employed surface zeta potential measurements of cells to confirm their use as a new tool to investigate the interactions of NPs and cells.[19]

EXPERIMENTAL PROCEDURES

Chemicals

Low-molecular-weight (LMW; molecular weight is 50,000–190,000 Da based on viscosity; viscosity 20–300 cps; deacetylation degree 75%–85%) and medium-molecular-weight (MMW; molecular weight is 190,000–310,000 Da based on viscosity 200–800 cps; deacetylation degree 75%–85%) water-soluble CS derived from crab shell, Tween 80, rhodamine B, and glacial acetic acid (*d* 1049 g/mL a 25°C) were purchased from Sigma–Aldrich (Milan, Italy). Sodium sulfate, sodium hydroxide, and all other chemicals were of analytical grade, purchased from Carlo Erba Reagents s.r.l (Milan, Italy). PLA and PLGA were purchased from Boehringer Ingheleim (Ingelheim am Rhein, Germany). Ultrapure water was used throughout this study.

For biological studies, we used OECs from 2-day-old rat pups (P2; Harlan, Bresso, Italy). Leibowitz L-15 medium, medium essential medium-H (MEM-H), collagenase and trypsin, Dulbecco's modified Eagle's medium (DMEM), foetal bovine serum (FBS), penicillin and streptomycin, and cytosine arabinoside were purchased from Sigma-Aldrich (Milan, Italy).

Chitosan Nanoparticles and Rhodamine-Loaded Nanoparticle Preparation

Low-molecular-weight (LMW) and medium molecular-weight (MMW) CS NPs were prepared according to a modified method [20] based on the ionic gelation of CS with sulfate anions. Utilizing an optimization procedure that we designed, a number of parameters were investigated by changing one parameter while keeping the others constant. These parameters included: types of CS, concentration of surfactant, stirring speed, sonication process, and time of sonication process. The optimization procedure was as follows: LMW or MMW CS was dissolved in an aqueous solution of acetic acid (1%, v/v) to form a 0.25 mg/mL CS solution. This solution was added with surfactant, Tween® 80, at different concentrations (0%, 0.5%, 1%, and 2%, w/v). The CS solution was stirred overnight at room temperature using a magnet stirrer. Na_2SO_4 was dissolved in ultrapure water at a concentration of 10% (w/v) and also passed through a syringe filter (pore size 0.22 μm, Millipore). To prepare CS NPs, Na_2SO_4 solution was added drop-by-drop into CS solution under a magnetic stirrer. The obtained suspension was underwent by sonication [Branson 5200, VWR (Milan, Italy] processed for 15 min. The sample was maintained under magnetic stirring for 1 h at a different stirring speed (500 and 1250 rpm). The obtained samples were reported in Table 1.

Table 1. Type of Chitosan (CS) and Variables Investigated for the Preparation of CS Nanoparticles of the Preliminary Screening

Batch	Type of CS	Tween® 80 (% w/v)	Time (min) of Sonication Process	Magnetic Stirrer (rpm)
NPCSLMW 1	Low molecular	0	0	500
NPCSLMW 2	weight	0.5	0	500
NPCSLMW 3	(LMW)	1	0	500
NPCSLMW 4		2	0	500
NPCSLMW 5		0	15	500
NPCSLMW 6		0.5	15	500
NPCSLMW 7		1	15	500
NPCSLMW 8		2	15	500
NPCSLMW 9		0	15	1250
NPCSLMW 10		0.5	15	1250
NPCSLMW 11		1	15	1250
NPCSLMW 12		2	15	1250
NPCSMMW 1	Medium	0	0	500
NPCSMMW 2	molecular	0.5	0	500
NPCSMMW 3	weight	1	0	500
NPCSMMW 4	(MMW)	2	0	500
NPCSMMW 5		0	15	500
NPCSMMW 6		0.5	15	500
NPCSMMW 7		1	15	500
NPCSMMW 8		2	15	500
NPCSMMW 9		0	15	1250
NPCSMMW 10		0.5	15	1250
NPCSMMW 11		1	15	1250
NPCSMMW 12		2	15	1250

Rhodamine B-loaded CS particles were prepared as previously reported, and the probe (0.005 mg/mL) was added before magnetic stirring so it was absorbed onto the particles surface.

Rhodamine-Loaded PLA and PLGA NPs Preparation

Nanoparticles obtained from PLA (NPPLA) and from PLGA (NPPLGA) were prepared by solvent displacement followed by polymer deposition. Briefly, the chosen polymer (75 mg) was dissolved in acetone (20 mL) with rhodamine B (0.005 mg/mL). The organic phase was added drop by drop under magnetic stirring into 40 mL of a water–ethanol solution (1:1, v/v) containing 0.5% (w/v) Tween® 80, obtaining a milky colloidal suspension. The organic solvent was then evaporated off under high vacuum at 40°C. The different formulations were purified from unloaded rhodamine B and surfactant by ultracentrifugation (15,000*g*) for 1 h at 10°C, using a Beckman (Fullerton, California) J2-21 model centrifuge equipped with a Beckman JA-20.01 fixed-angle rotor. After washing, the obtained NPs were re-suspended in 5 mL of filtered water (0.22 μm Sartorius membrane filters) and characterized for size distribution and surface chemistry.

Characterization of NPs: Size and Zeta Potential Evaluation

The mean particle size (*Z*-average) and the polydispersity index (PDI) were determined at 25°C by PCS using a Zetasizer Nano ZS (Malvern, Malvern, UK; laser 4 mW He–Ne, 633 nm, laser attenuator automatic, transmission 100%–0.0003%, detector avalanche photodiode, Q.E. > 50% at 633 nm). The *Z*-potential was measured using the same equipment with a