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Non-viral vectors based on magnetoplexes, lipoplexes and polyplexes for VEGF gene delivery into central nervous system cells

Ilia Villate-Beitia[a,b], Gustavo Puras[a,b], Cristina Soto-Sánchez[b,c], Mireia Agirre[a,b], Edilberto Ojeda[a,b], Jon Zarate[a,b], Eduardo Fernández[b,c], José Luis Pedraz[a,b,*]

[a] NanoBioCel Group, University of the Basque Country (UPV/EHU), Vitoria-Gasteiz, Spain
[b] Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain
[c] Neuroprothesis and Neuroengineering Research Group, Miguel Hernandez University, Elche, Spain

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ABSTRACT

Nanotechnology based non-viral vectors hold great promise to deliver therapeutic genes into the central nervous system (CNS) in a safe and controlled way. Vascular endothelial growth factor (VEGF) is a potential therapeutic gene candidate for CNS disorders due to its specific roles in brain angiogenesis and neuroprotection. In this work, we elaborated three different non-viral vectors based on magnetic, cationic lipid and polymeric nanoparticles complexed to the phVEGF165aIRESGFP plasmid, which codifies the VEGF protein –extracellular- and the green fluorescent protein (GFP) –intracellular-. Nanoparticles and corresponding nanoplexes –magnetoplexes, lipoplexes and polyplexes- were characterized in terms of size, zeta potential, polydispersity index, morphology and ability to bind, release and protect DNA. Transfection efficiencies of nanoplexes were measured in terms of percentage of GFP expressing cells, mean fluorescent intensity (MFI) and VEGF (ng/ml) production in HEK293, C6 and primary neuronal culture cells. Magnetoplexes showed the highest transfection efficiencies in C6, followed by lipoplexes, and in primary neuronal culture cells, followed by polyplexes. Lipoplexes were the most efficient in HEK293 cells, followed by magnetoplexes. The biological activity of VEGF was confirmed by its proliferative effect in HUVEC cells. Overall, these results provide new insights for VEGF gene delivery into CNS cells using non-viral vectors.

1. Introduction

Nanotechnology-based gene delivery has gained interest in recent years. Continuous advances in the fields of material science and nano-engineering – along with a better understanding of the multiple possibilities for genome editing – have motivated the synthesis, characterization, and functionalization of biocompatible nanomaterials for gene delivery purposes (Keles et al., 2016). In addition, the diversity of available nanosized material allows the design of multifunctional vectors specifically tailored for different applications (Srikanth and Kessler, 2012; Pezzoli et al., 2012).

Non-viral vectors are characterized by their potential to overcome many of the limitations of viral vectors, especially those related to safety and vector production. Although viral vector based gene delivery platforms are still predominant in clinical trials due to their higher transfection efficiencies (Yin et al., 2014), non-viral vectors have emerged as a promising alternative because of their lower immunogenicity, higher nucleic acid packing capacity and ease of fabrication (Jin et al., 2014). Additionally, non-viral formulations can be produced on a large scale with high reproducibility and acceptable costs, and they are relatively stable to storage (Pezzoli et al., 2012). To date, a wide variety of nanosized non-viral gene delivery vectors have been developed, including cationic lipids (Yin et al., 2014; Ojeda et al., 2015; Jubeli et al., 2016), polymers (Pack et al., 2005; Agirre et al., 2015a) and magnetic nanoparticles (Soto-Sánchez et al., 2015). These molecules can be nanoengineered to transport therapeutic genes into specific organs or cell types and to pass over several extracellular and intracellular barriers.

Central nervous system (CNS) cells are one of the most challenging to transfect for both viral and non-viral gene delivery systems, due to the numerous protective barriers enclosing the CNS and its complex cellular organization. Therefore, the discovery of an effective approach to afford the wide range of

* Corresponding author at: Laboratory of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of the Basque Country, 01006, Vitoria-Gasteiz, Spain.
E-mail address: joseluis.pedraz@ehu.eus (J.L. Pedraz).

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neurodegenerative disorders remains a huge dare, where nanotechnologies are expected to play an important role. Most neurodegenerative disorders, although presenting diverse clinical manifestations, share some common traits such as vast neuronal loss, synaptic failures and aggregates of misfolded proteins (Spuch et al., 2012). Additionally, accumulating evidence suggests that aging-related vascular alterations of the brain make it vulnerable to cognitive dysfunction, leading to neurodegeneration and dementia (Kalaria, 2010). In Alzheimer's disease (AD) – the most prevalent adult-onset dementia – reductions in the cerebral blood flow and dysfunction of the blood-brain barrier (BBB) have been related to amyloid-beta (Aβ) accumulation in brain (Zlokovic, 2011). In this regard, vascular endothelial growth factor (VEGF) is a physiological regulator mainly involved in brain angiogenesis and BBB integrity, and it also plays specific roles in neuroprotection (Carmeliet and Ruiz de Almodovar, 2013a). Recently, it has been shown that administration of VEGF encapsulated in poly(lactic-co-glycolic acid) (PLGA) nanospheres to animal models of AD increases vascularisation and reduces neuronal loss and Aβ deposits in brain (Herrán et al., 2013a). Also, it has been reported that, in a mouse model of AD, administration of encapsulated VEGF-secreting cells enhances proliferation of neuronal progenitor cells in the hippocampus (Antequera et al., 2012).

Based on these considerations, we present a nanoformulation based approach for VEGF gene delivery into CNS cells. For that purpose, three different nanoformulations –ultrapure oligochitosan (UOC), *Lipofectamine* cationic lipid and *NeuroMag* magnetic nanoparticles– were complexed to the phVEGF165aIRESGFP plasmid, which codifies for the human VEGF165a protein and for the green fluorescent protein (GFP). Nanoformulations and resulting nanoplexes –nanoformulations complexed to DNA– were physicochemically characterized in terms of size, superficial charge, polydispersity index (Đ), morphology and capacity to condense, release and protect DNA. *In vitro* transfection studies were carried out in C6 glial cells and in primary neuronal culture cells as CNS cell models, and in HEK293 cells, as a general transfection model. Transfection efficiencies were evaluated in terms of percentage of GFP expressing cells, mean fluorescent intensity (MFI) and VEGF protein expression. Cell viability upon exposition to nanoplexes was also evaluated. Bioactivity of the VEGF protein secreted by transfected primary neuronal culture cells was assessed by a proliferation assay in human umbilical vein endothelial cells (HUVEC).

2. Materials and methods

2.1. Materials

Ultrapure Oligochitosan (UOC) O15 (MWs of 5.7, DDA ≥97% and endotoxin levels ≤0.05 EU/mg) was purchased from NovaMatrix/FMC (Sandvika, Norway). Human embryonic kidney 293 (HEK293) cells, Rattus Norvegicus glial cells (C6), human umbilical vein endothelial cells (HUVEC), Eagles's Minimal Essential Medium with Earle's BSS and 2 mM L-glutamine (EMEM, ATCC 30-2003) and Kaighn's Modification of Ham's F-12 Medium (F-12 K, ATCC) were obtained from the American Type Culture Collection (ATCC, Teddington, UK). Endothelial Cell Growth Medium (EGM-2 Bullekit, CC-3162) was purchased from Lonza Group Ltd. (Basel, Switzerland). Opti-MEM I reduced medium, Antibiotic/Antimicotic solution, Penicillin/Streptomycin solution and Lipofectamine 2000 were purchased from Invitrogen (Life Technologies, Paisley, UK). The phVEGF165aIRESEGFP plasmid was purchased from Plasmid-Factory (Bielefeld, Germany). The Human VEGF ELISA Development Kit and the recombinant hVEGF165 protein were provided by Peprotech EC Ltd. (London, UK). Phosphate buffer saline (PBS), fetal bovine serum (FBS), horse serum (HS), Cell Counting Kit-8 (CCK-8), DNase I, sodium dodecyl sulfate (SDS) and propidium iodide were acquired from Sigma-Aldrich (Madrid, Spain). Gel electrophoresis materials were obtained from Bio-Rad (Madrid, Spain). NeuroMag Transfection Reagent was acquired from OZ Biosciences (Marseille, France). TECAN infiniteM200 spectrophotometer was acquired from TECAN Ibérica Instrumentación S.L., Madrid, Spain.

2.2. Preparation of nanoplexes

Lipoplexes were prepared at a Lipofectamine/DNA ratio of 2:1 (w/w) by mixing gently Lipofectamine 2000 solution (1 mg/ml) and the plasmid solution (1 mg/ml) at appropriate volumes. Magnetoplexes were prepared at a NeuroMag/DNA ratio of 2:1 (w/w) by mixing gently NeuroMag solution (1 mg/ml) and plasmid solution (1 mg/ml) at appropriate volumes. Polyplexes based on UOC were prepared by the self-assembly method. Briefly, a determined value of plasmid solution (1 mg/ml) was added under vortex agitation to a stock solution of UOC (2 mg/ml) to obtain a final UOC/DNA ratio of 13:1 (w/w). In all cases, the formulations were incubated for 25 min at room temperature to allow the correct formation of the complexes.

2.3. Nanoformulation characterization: size, zeta potential, Đ, morphology

The hydrodynamic diameter and the zeta potential of nanoparticles and their corresponding nanoplexes were determined by Dynamic Light Scattering (DLS) and by Lasser Doppler Velocimetry (LDV), respectively, using a Zetasizer Nano ZS (Malvern Instrument, UK) as previously described (Ojeda et al., 2015). Briefly, 50 μl of the formulations were resuspended into 950 μl of 0.1 mM NaCl solution. All measurements were carried out in triplicate. The particle size reported as hydrodynamic diameter was obtained by cumulative analysis. The Smoluchowski approximation was used to support the calculation of the zeta potential from the electrophoretic mobility.

The morphologies of cationic lipid and magnetic nanoparticles were characterized by transmission electron microscopy (TEM) was used to characterize the morphology of UOC polymeric nanoparticles as previously described (Ojeda et al., 2015).

2.4. Agarose gel electrophoresis assay

For DNA release assay, 12 μl of a 7% SDS solution were added to the samples. For DNA protection assay, 3 μl of DNase I enzyme and 12 μl of a 7% SDS solution were added to the samples. For DNA binding assay, no SDS neither DNase I enzyme were added to the samples. The amount of DNA per well was 100 ng in all cases. Samples with DNase I were incubated at 37 °C for 30 min. 4 μl of loading buffer were added to all samples before running the gel. Agarose gel (0.8% w/w) was immersed in a Tris-acetate-EDTA buffer and exposed for 30 min to 120 V. GelRed was used to stain the DNA bands and images were obtained with a ChemiDocTM MP Imaging System and analyzed by Image LabTM Software (BioRad, USA). Naked DNA was used as control at each condition.

2.5. Cell culture and in vitro transfection

HEK293 and C6 cells were maintained in complete medium. For HEK293 cells, Eagles's Minimal Essential medium with Earle's BSS and 2 mM 1-glutamine (EMEM, ATCC 30-2003) supplemented with 10% FBS and Antibiotic/Antimycotic solution was used. For C6 cells, Kaighn's Modification of Ham's F-12 Medium (F-12 K, ATCC 30-2004) supplemented with 2.5% FBS, 15% HS and Penicillin/Streptomycin solution was used. Both cell cultures were kept at 37 °C under a humidified atmosphere containing 5% CO_2. Before