International Journal of Pharmaceutics 516 (2017) 39–44

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Improved formulation of cationic solid lipid nanoparticles displays cellular uptake and biological activity of nucleic acids

Anna Fàbregas[a,1], Silvia Prieto-Sánchez[b,1], Marc Suñé-Pou[a,b,1], Sofía Boyero-Corral[b], Josep Ramón Ticó[a,c], Encarna García-Montoya[a,c], Pilar Pérez-Lozano[a,c], Montserrat Miñarro[a,c], Josep Mª Suñé-Negre[a,c], Cristina Hernández-Munain[d], Carlos Suñé[b,*]

[a] Drug Development Service, Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Barcelona, Avda. Joan XXIII, s/n 08028 Barcelona, Spain
[b] Department of Molecular Biology, Institute of Parasitology and Biomedicine "López Neyra" (IPBLN-CSIC), PTS, Granada 18016, Spain
[c] Bellvitge Biomedical Research Institute (IDIBELL), University of Barcelona, Spain
[d] Department of Cell Biology and Immunology, Institute of Parasitology and Biomedicine "López Neyra" (IPBLN-CSIC), PTS, Granada 18016, Spain

ARTICLE INFO

Article history:
Received 13 September 2016
Received in revised form 7 November 2016
Accepted 9 November 2016
Available online 10 November 2016

Chemical compounds studied in this article:
Stearic acid (PubChem CID: 5281)
Octadecylamine (PubChem ID: 15793)
Poloxamer 188 (PubChem CID: 24751)

Keywords:
Cationic solid lipid nanoparticles (SLNs)
Plasmid DNA
siRNA
Binding efficiency
Transfection
Luciferase

ABSTRACT

Non-viral delivery using cationic solid lipid nanoparticles (SLNs) represents a useful strategy to introduce large DNA and RNA molecules to target cells. A careful selection of components and their amounts is critical to improve transfection efficiency. In this work, a selected and optimized formulation of SLNs was used to efficiently transfect circular DNA and linear RNA molecules into cells. We characterized the main physicochemical characteristics and binding capabilities of these SLNs and show that they deliver DNA and RNA molecules into cells where they display full bioactivity at nontoxic concentrations using fluorescence- and luminescence-based methodologies. Hence, we established a novel and simple SLN formulation as a powerful tool for future therapeutic use.

1. Introduction

In recent decades, the transfection of nucleic acids using nanostructured non-viral delivery systems has become a powerful strategy in nanomedicine to achieve gene expression regulation for the treatment of diseases, thus representing a promising new avenue for gene therapy (Chang et al., 2011; Ding et al., 2014; Uchida et al., 2016; Wang et al., 2015). The advantages of non-viral gene delivery systems compared to viral systems have been widely reported and are clear in terms of the low immunogenic response generated from suitable compositions, characteristics, and behaviours of the designed nanostructures (Nayerossadat et al., 2012; Severino et al., 2015). These properties avoid possible host immune responses that could jeopardize the success of the proposed therapy. Whereas low immunogenicity is as a positive trait, one obstacle of non-viral delivery systems is that efficient and reliable transfection efficiency, which is a hallmark of viral systems that have continuously evolved through many years of evolutionary improvement, must be achieved. This limitation can be due to several factors that range from the composition of the delivery system and the cell membrane characteristics, especially the presence of proteoglycans, to the cell line features and regulatory mechanisms that may elicit different responses for the same gene delivery system. The characteristics of the nucleic acid to be delivered are also important features to consider, specifically the size and its folding, which may affect the number and exposure of negative charges in its backbone structure for binding to nanoparticles to form lipoplexes. For instance, a plasmid DNA (pDNA) behaves differently than a small linear RNA. Whereas the former is

* Corresponding author.
E-mail address: csune@ipb.csic.es (C. Suñé).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ijpharm.2016.11.026
0378-5173/

usually thousands of base pairs long, the latter does not exceed 25 base pairs in length. Considering size only, it is expected that short RNA molecules are easier to transfect into cells than large pDNA molecules. Intracellular trafficking and processing upon nucleic acid transfection are also key steps that may impact the final yield in terms of the biological activity. Nonetheless, there is a need for *in vivo* pDNA and RNA delivery through complexation with nanoparticles; this need has attracted government funding and commercial interest in the pharmaceutical industry (Chang et al., 2015; Kraljevic and Pavelic, 2005).

Cationic solid lipid nanoparticles (SLNs) are one of the most promising nanoparticle systems for nucleic acid delivery for therapeutic usage (Carrillo et al., 2013; Moritz and Geszke-Moritz, 2015; Rostami et al., 2014; Yu et al., 2012). This type of nanostructure is based in a lipid formulation and can be prepared by several methods (Muller et al., 2000). SLNs have several advantages compared to other nanoparticle systems. Among these systems, the use of biocompatible lipids may decrease the toxicity of the nanoparticles, therefore improving cell tolerance to treatment; their manufacturing process allows efficient scaling for large-scale production with a good cost-effective ratio (Muller et al., 2000). However, a main drawback of SLNs (and nanoparticles in general) that may hinder the implementation of these systems for therapeutic purposes is the very complex formula for keeping nanoparticles stabilized. Current research in this field aims to overcome this problem and develop effective nanoparticle systems for delivering nucleic acids while maintaining the advantages of viral vectors (Pozzi et al., 2014).

In a previous work, we showed that SLNs are capable of forming complexes with DNA plasmids with low toxicity when assayed in human cells (Fabregas et al., 2014). This manuscript builds on these studies to obtain SLNs from an optimized formula that maintains the structure, morphology, and nucleic acid binding efficiency with minimum cell toxicity that is able to enter different cell lines and that release nucleic acids to induce protein expression and biological activity.

2. Materials and methods

2.1. Production of SLNs

The SLNs were produced using stearic acid (Merck Millipore), octadecylamine (Across Organics, USA), Poloxamer 188 (Sigma-Aldrich, USA) and ultrapure water (Merck Millipore) as previously described (Fabregas et al., 2014). The approximately 6.5 kilobase pEGFP plasmid that contains enhanced green fluorescent protein sequences in the pEF-BOS vector (Mizushima and Nagata, 1990), and the approximately 6.0 kilobase p3X-κB-L DNA plasmid that contains the cDNA encoding the Firefly luciferase protein (Mitchell and Sugden, 1995), were purified with Qiagen kits (Hilden, Germany). A dual luciferase assay kit (Promega, USA) was used for the detection of luciferase activity. The RNA that was used was a 21-bp double-stranded oligo (Isogen Life Sciences, Netherlands) carrying sequences from the human transcription and splicing factor TCERG1 (Montes et al., 2012) modified with Cy3 fluorophore at 5′ of the sense chain. HEK293T and HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco), L-glutamine (Gibco) at 4 mM, and penicillin/streptomycin (Gibco) to 100 units and 100 μg per ml, respectively.

The SLNs were synthesized with the microemulsification method using stearic acid, octadecylamine and Poloxamer 188 (5:6:1) (Fabregas et al., 2014). All the assays except for the stability studies were performed with lyophilized SLNs (L-3 Telstar, Spain) using trehalose (5% w/v) as a cryoprotectant.

2.2. Determination of particle size

The size of the nanoparticles was determined by laser diffraction technique according to the Mie theory on a Mastersizer 2000 (Malvern Instruments, UK) directly from the suspension of SLNs. The particle size was determined in triplicate, and the mean value was calculated in nanometers (nm).

2.3. Determination of surface charge (zeta-potential)

The surface potential of the cationic nanoparticles was determined by laser *Doppler* micro electrophoresis in a Zetasizer Nano-Z (Malvern Instruments, UK). The zeta-potential values were obtained from the electrophoretic mobility of the nanoparticles under an electric field. The measurements were made in triplicate and expressed as millivolts (mV).

2.4. Morphological analysis

The surface and content homogeneity of the nanoparticles was analyzed through scanning and transmission electron microscopy. Images were acquired from reconstituted SLNs after lyophilizing with trehalose 5% w/v as a cryoprotectant. The scanning electron microscopy (SEM) images were obtained using a Hitachi S-4300 microscope (Japan) at 10 keV on previously conditioned samples that were sputter-coated with a carbon film. The transmission electron microscopy (TEM) images were obtained using a JEOL JEM 2100 LaB6 microscope (Japan) at 200 kV. The samples were previously air-dried on a holey carbon grid.

2.5. Thermal stability

The stability of newly synthesized SLNs was examined by preparing the samples in hermetically sealed glass vials at 4 °C (LG, South Korea), 25 °C, and 37 °C (Heraeus, Germany). The 37 °C condition, corresponding to physiological temperature, is considered a possible indicator for biocompatibility. The particle size, and Z potential were analyzed over 7 days for each of the conditions tested.

2.6. Formation of lipoplexes

Formation of SLNs-nucleic acid complexes was carried out as previously described with minor modifications (Fabregas et al., 2014). Briefly, different volumes of a SLN suspension were mixed with fixed amounts of pDNA or RNA to determine the volume of nanoparticles that is best able to retain the maximum pDNA or RNA. After a 45 min incubation period at room temperature, the binding capacity of the SLNs was determined via the electrophoretic mobility of the lipoplexes in agarose gels.

2.7. Gel retardation assay

For the DNA electrophoretic gel separations, we used D-1 Media EEO agarose in 1 x TAE buffer (40 mM Tris acetate, 1 mM EDTA) and 0.5 μg/ml of ethidium bromide (EtBr) for nucleic acid visualization. The loading buffer was 0.25% (w/v) bromophenol blue, 0.25% (w/v) xilenocianol FF, and 30% (v/v) glycerol. The power sources, cuvettes, and accessories were all from BioRad. The electrophoretic conditions were set at a voltage of 75 V for 45 min. The samples were visualized in a Gel Doc station (BioRad) using QuantityOne software. The data shown are representative of at least three independent experiments.