European Journal of Pharmaceutical Sciences 49 (2013) 157–165

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutical Sciences

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

DNA delivery via cationic solid lipid nanoparticles (SLNs)

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ARTICLE INFO

Article history:
Received 21 May 2012
Received in revised form 17 January 2013
Accepted 4 February 2013
Available online 27 February 2013

Keywords:
Non-viral vectors
Lipoplexes
Gene therapy
Solid lipid nanoparticles
Cationic nanoparticles
Drug delivery systems

ABSTRACT

In recent years the use of solid lipid nanoparticles (SLNs) as transport systems for the delivery of drugs and biomolecules has become particularly important. The use of cationic SLNs developed by the technique of microemulsion, which are complexed with DNA in order to study their application as non-viral vectors in gene therapy, is reported. The nanoparticles are characterized by scanning electron microscopy and transmission electron microscopy (SEM and TEM), atomic force microscopy (AFM) and differential scanning calorimetry (DSC). Furthermore, the process of lyophilization of the samples and their stability was studied. The nanoparticles obtained presented a particle size of 340 nm with a positive surface charge of 44 mV and the capability of forming lipoplexes with DNA plasmids was stated.

1. Introduction

Nanoscience and nanotechnology have received much attention in the last decade and they now form one of the most important fields of technology and innovation. It is a field that has attracted the attention of both industry and governments around the world (Dowling, 2004; Zweck et al., 2008; Aguar Fernandez and Hullmann, 2007).

Due to rapid progresses in nanotechnology and biotechnology, nanoparticles have come to be seen as a viable vehicle for the delivery and release of drugs. Nanoparticles have thus advanced rapidly in the pharmaceutical world and their production and application in biomedicine has become one of the most important developments in nanotechnology and nanoscience (Nowack and Bucheli, 2007; Medina et al., 2007; Wong et al., 2012).

Within this field, the use of solid lipid nanoparticles (SLNs) is of particular importance. SLNs comprise a variety of systems with a particle diameter of between 50 and 1000 nm, and their proprieties make them a viable alternative to polymeric systems (Battaglia et al., 2010; Mehnert and Mäder, 2012). They are colloidal particles made up of a relatively rigid biocompatible and biodegradable matrix of hydrophobic lipids that are solid at room and body temperatures.

SLNs have experienced constant development over recent years as drug delivery systems, although there is not much literature concerning their application in gene therapy. Their capacity to transfect cells *in vitro* has been demonstrated (Olbrich et al., 2001; Tabatt et al., 2004; del Pozo-Rodríguez et al., 2008, 2010; Martins et al., 2012) since the 1990s when Müller (1991) and Gasco (1993) first studied the proprieties of SLNs in drug delivery.

SLNs were originally designed as an alternative to liposomes and emulsions. Although liposomes present a series of advantages (encapsulation of both hydrophilic and hydrophobic active ingredients, reduced toxicity and increased therapeutic efficiency of active ingredients that is not achieved with other systems), their application has seen only limited success (Borchard, 2001). Such limitations are due to the complexity associated with the process of obtaining liposomes, scaling difficulties, their limited stability and the high cost of their formulation (Joshi and Müller, 2009). SLNs have the advantages of colloidal systems of drug delivery, such as liposomes, polymeric nanoparticles and emulsions, while at the same time they considerably minimize or reduce the inconveniences associated with these systems. Some of their advantages include the ability to integrate both hydrophilic and hydrophobic drugs as well as the ability to prolong active ingredient release or to immobilize it in the solid matrix. These SLNs properties compared to polymeric nanoparticles are based on their low cytotoxicity, high capacity for transfection, better stability in biological

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http://dx.doi.org/10.1016/j.ejps.2013.02.011

systems and improved scalability (Blasi et al., 2007; Wissing et al., 2004; Marengo et al., 2000; Müller et al., 2000; Wang et al., 2012).

Gene therapy is the set of techniques by means of which fragments of DNA or RNA can be delivered to the inside of specific cells in order to modulate the expression or suppression of the biosynthesis of specific altered proteins so as to reverse a biological disorder and treat disease (Ledley, 1996; Corsi et al., 2003).

Gene delivery systems include both viral and non-viral vectors. A wide variety of vectors for the delivery of genetic material have been studied (Nishikawa and Hashida, 2002). Viral vectors are one of the strategies more generally used and are the dominant systems for gene delivery with high transfection efficiency. However, viral vector applications are limited due to the adverse effects associated with them such as their immunogenicity and oncogenicity (Wu and Ataai, 2000; Richardson et al., 1999; De Laporte et al., 2006). These limitations have led to the development of effective synthetic systems for delivering DNA incorporating the DNA in nanoparticles while aiming to maintain the advantages of viral vectors.

A large number of non-viral vectors have been studied and applied as non-natural systems of stable transfection that contain low toxicity and are non-immunogenic since they can auto-assemble with the DNA and form nanoparticles capable of being transported to the cells (Davis, 2002; He et al., 2010; Ewert et al., 2005; Li and Huang, 2007; Ferrer-Miralles et al., 2008; Rogers and Rush, 2012; Liang et al., 2012).

Non-viral vectors include cationic lipids. The electrostatic interaction between the negative charges of the DNA and the positive charges of the lipid allow the formation of a complex called lipoplex. These lipoplexes can form a structure that protects the DNA and which is able to direct it towards the target cells (Pedersen et al., 2006; Faneca et al., 2002; del Pozo-Rodríguez et al., 2007; Duarte et al., 2012).

Our purpose is to develop a method for obtaining cationic SLNs capable of forming a complex with DNA plasmids. A series of characteristics for the particles to be considered good non-viral vector are required. On the one hand, they must be nanometric in size with a suitable surface charge to form complexes with DNA plasmids, and on the other hand they must be stable.

2. Materials and methods

2.1. Materials

Stearylamine (Sigma–Aldrich®, Barcelona, Spain), 2-methyloxirane as a hydrophilic non-ionic surfactant (Poloxamer® 188-BASF®, Germany), glycerol distearate (Precirol® ATO-5-Gattefosé®, France), trehalose (Cerestar®, Barcelona, Spain), mannitol (Fagron®, Barcelona, Spain). The media used are DMEM (Dulbeco's Modified Eagle's Medium, PAA® Laboratories, Austria), DMEM supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml) (Invitrogen®) and HBS (Hepes buffered saline, Sigma–Aldrich®, Barcelona, Spain). The plasmid DNA used is pMS2-TCERG1 plasmid of approximately seven kilobases that contains the cDNA encoding the carboxyl region of the human transcription factor TCERG1 (Suñé et al., 1997), purified with Qiagen® kits (Hilden®, Germany).

2.2. Methods

2.2.1. Obtaining the SLNs

To obtain cationic SLNs, a modification of the method described has been used (Vighi et al., 2007; Bondi' et al., 2007). The SLNs are obtained from a microemulsion (O/W) using Precirol ATO-5 and stearylamine as the cationic lipid. 500 mg of Precirol ATO-5 is heated to 10 °C above its melting point, and 10 ml of a hot aqueous solution of poloxamer and stearylamine in different proportions (1/1.25; 1/1.87; 1/3.12; 1/4.37 and 1/5 respectively) is added. The sample is stirred for 30 min at 14,000 rpm (IKA® T25 digital Ultra-Turrax®, Staufen, Germany). The nanoparticles are obtained by dispersing the hot microemulsion in cold water (between 2 °C and 5 °C) in an emulsion:water ratio of 1:5. To recover nanoparticles, the resultant suspension is centrifuged for three times at 3000 rpm for 20 min at a temperature of 20 °C, reconstituting the precipitate after centrifugation. Part of the fresh sample is then stored at 4 °C, while the other part of the sample is lyophilized.

2.2.2. Lyophilization of samples

Cationic SLNs are lyophilized by being added an aqueous solution of cryoprotectant in the proportion 1:2 (SLN:cryoprotectant). Mannitol and trehalose are the cryoprotectants used in the study (of 5% and 10% in both cases). The freezing temperature is set at −40 °C in the lyophilizer Telstar® L-3 (Telstar®, Terrassa, Spain), and samples are kept at this temperature for 2 h. Lyophilization temperature is then set to 25 °C at a pressure of 0.2–0.4 mBa for 48 h.

2.2.3. Determination of particle size

The distribution of the particle size of the cationic SLNs is determined by laser diffraction technique (Mastersizer® 2000, Malvern Instruments®, UK), by dispersing the sample with bidistilled water in the Hydro dispersion module (Malvern Instruments®, UK). Particle size is determined in triplicate, and the mean value is calculated. The size data are evaluated using the following distribution volumes: d10%, d50% and d90% (European Pharmacopoeia, 2012).

2.2.4. Determination of surface charge

Particles zeta-potential values are determined by using the light dispersion technique with Zetasizer® Nano Z equipment (Malvern Instruments®, UK). An amount of sample is placed in the capillary cell for samples in aqueous media at a predetermined temperature of 25 °C. Surface charge is measured three times and the mean value taken.

2.2.5. Electron microscopy and atomic force microscopy (AFM)

The morphology of the cationic SLNs is studied by electron microscopy both scanning electron microscopy (Hitachi® S-2300, Japan) and transmission electron microscopy (Hitachi® 800MT, Japan), and in this way their shape and size are characterized.

Atomic force microscopy (AFM) is used to study the topography of the samples: their morphology and their particle size. Extended Multimode equipment is used for the AFM with Nanoscope® IV (Veeco®, Mannheim, Germany) and images are captured in Peak Force mode. A silicon nitride cantilever probe with silicon oxide tips of 0.35 nN/nm (SNL-10, Bruker®, USA) is used.

2.2.6. Differential scanning calorimetry (DSC)

The thermal stability of the compounds is studied using the differential scanning calorimetry (DSC) equipment DSC-822e/700 (Mettler Toledo®, Spain) at temperatures of between 30 °C and 300 °C for 30 min (10 °C/min). Between 2 and 5 mg of each of the components of the formulation is individually subjected to DSC, together with binary and tertiary mixtures of them, and also both lyophilized and fresh (non-lyophilized) SLNs.

2.2.7. Stability

The study of stability is carried out by preparing the samples in hermetically sealed glass vials at a temperature of 30 °C ± 2 °C. Samples' morphology and appearance are then analyzed together with the pH and the superficial charge of the nanoparticles at different times: $t = 0$; 24 h; 10 days and 30 days in order to detect physical or chemical changes (Mora-Huertas et al., 2010). The