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Design of cationic lipid nanoparticles for ocular delivery: Development, characterization and cytotoxicity

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ABSTRACT

In the present study we have developed lipid nanoparticle (LN) dispersions based on a multiple emulsion technique for encapsulation of hydrophilic drugs or/and proteins by a full factorial design. In order to increase ocular retention time and mucoadhesion by electrostatic attraction, a cationic lipid, namely cetyltrimethylammonium bromide (CTAB), was added in the lipid matrix of the optimal LN dispersion obtained from the factorial design. There are a limited number of studies reporting the ideal concentration of cationic agents in LN for drug delivery. This paper suggests that the choice of the concentration of a cationic agent is critical when formulating a safe and stable LN. CTAB was included in the lipid matrix of LN, testing four different concentrations (0.25%, 0.5%, 0.75%, or 1.0%wt) and how composition affects LN behavior regarding physical and chemical parameters, lipid crystallization and polymorphism, and stability of dispersion during storage. In order to develop a safe and compatible system for ocular delivery, CTAB-LN dispersions were exposed to Human retinoblastoma cell line Y-79. The toxicity testing of the CTAB-LN dispersions was a fundamental tool to find the best CTAB concentration for development of these cationic LN, which was found to be 0.5 wt% of CTAB.

1. Introduction

Lipid nanoparticles (LN) have gained interest in recent years as drug carriers for ocular delivery, aiming a better permeation and/or prolonged drug release onto the ocular mucosa and allowing drugs reaching the post segment of the eye (Pignatello and Puglisi, 2011). Ocular drug delivery is extremely affected by eye anatomy and physiology that leads often to mechanisms that decrease bioavailability of applied drugs. These mechanisms include reflex processes, such as lacrimation and blinking which reduces drastically the drug residence time, and difficulty to diffuse though the conjunctiva and nasolacrimal duct. In addition, the low volume of the conjunctival sac also leads to a poor corneal or sclera penetration of drugs (Diebold and Calonge, 2010). Since ocular delivery became a problem when the ultimate target is intraocular delivery, due to the ineffective drug concentrations and time residence reach the inner tissues, alternative systems for drug delivery are required (Pignatello and Puglisi, 2011; Sultana et al., 2011). New drug delivery systems based on lipids, namely liposomes, and other materials such as polymers (poly-D-L-lactic acid (PLA) nanopsheres) were able to deliver an antiviral drug, acyclovir, in the inner tissues of the eye comprising the innovation of these systems (Fresta et al., 1999; Giannavola et al., 2003).

Ocular drug delivery strategies may be classified into 3 groups: noninvasive techniques, implants, and colloidal carriers. Colloidal drug delivery systems, such as LN, can be easily administered in a liquid form and have the ability to diffuse rapidly and are better internalized in ocular tissues. In addition, the interaction and adhesion of LN ocular surface with the endothelium makes these drug delivery systems interesting as new therapeutic tools in ocular delivery (del Pozo-Rodriguez et al., 2013).

LN based on w/o/w emulsion are versatile colloidal carriers for the administration of peptides/proteins and hydrophilic drugs (Fangueiro et al., 2012). Droplets from the inner aqueous phase,

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where the drug is dissolved or/and solubilized, are supported by a solid lipid matrix surrounded by an aqueous surfactant phase. Usually LN are composed of physiological solid lipids (mixtures of mono-, di- or triglycerides, fatty acids or waxes) stabilized by surfactants. In the case of a w/o/w based LN dispersion, a high hydrophilic–lipophilic balance (HLB) surfactant is added to the external aqueous phase and, a low HLB surfactant is added to the lipid phase. The two surfactants are needed to stabilize the two existing interfaces in this type of emulsion. A variety of surfactants can be applied, such as phospholipids, bile salts, polysorbates, polyoxyethylene ethers (Gallarate et al., 2009; Fangueiro et al., 2012). Materials used for LN production are largely used in pharmaceutical industry with proved biocompatibility (Severino et al., 2012).

Cationic LN have been recently investigated for targeting ocular mucosa, namely the posterior segment of the eye (e.g. retina). This is a smart strategy that combines the positive surface charge of the particles and the negative surface charge of ocular mucosa by means of an electrostatic attraction. This approach could increase the drugs retention time in the eye as well as improve nanoparticles bioadhesion (Lallemand et al., 2012).

In the ocular delivery, it is especially relevant the control of the particle size since it directly influence the drug release rate, bioavailability, and patient comfort and compliance (Shekunov et al., 2007; Souto et al., 2010). In addition, it is known that the smaller the particle size, the longer the retention time and easier application (Araujo et al., 2009).

Physicochemical characterization and assessment of nanotoxicity are major issues for developing and large-scale manufacturing of nanocarriers. Furthermore, physicochemical properties of LN such as particle size, surface and composition can significantly influence drug delivery on ocular delivery (Ying et al., 2013).

In the present work, the development and characterization of a system of LN based on multiple emulsions using a blend of triacylglycerols as solid lipid, was carried out, in which sonication method was employed. The first aim of the work was the application of a full factorial design to determine which dependent variables could affect the LN dispersion properties. The analyzed independent variables, namely the concentration of solid lipid and both hydrophilic and lipophilic surfactants, were checked for their capacity to influence the mean particle size (Z-Ave), polydispersity index (PI) and zeta potential (ZP) of the produced LNs dispersions. The optimal formulation was used to evaluate the toxicity of LN using Y-79 human retinoblastoma cells employing different cationic lipid concentrations to select the best formulation for ocular instillations.

2. Materials and methods

2.1. Materials

Softisan® 100 (S100, a hydrogenated coco-glycerides C_{10}–C_{18} fatty acid triacylglycerol) used as solid lipid was a free sample from Sasol Germany GmbH (Witten, Germany), Lipoid® S75, 75% soybean phosphatidylcholine, used as surfactant, was purchased from Lipoid GmbH (Ludwigshafen, Germany), Lutrol® F68 or Poloxamer 188 (P188) was a free sample from BASF (Ludwigshafen, Germany). Cetyltrimethylammonium bromide (CTAB) and uranyl acetate were acquired from Sigma–Aldrich (Sintra, Portugal). Anhydrous glycerol was purchased from Acopharma (Barcelona, Spain). Ultra-purified water was obtained from a MiliQ Plus system (Milipore, Germany). All reagents were used without further treatment. The Y-79 human retinoblastoma cell line was purchased from Cell Lines Service (CLS, Eppelheim, Germany). Reagents for cell culture were from Gibco (Alfagene, Invitrogene, Portugal).

Table 1
Initial 3-level full factorial design, providing the lower (−1), medium (0) and upper (+1) level values for each variable.

Variables	Levels		
	Low level (−1)	Medium level (0)	High level (+1)
S100 (wt%)	2.5	5.0	7.5
Lecithin (wt%)	0.25	0.5	0.75
P188 (wt%)	0.5	1.0	1.0

S100: Softisan® 100; P188: Poloxamer 188.

2.2. Experimental factorial design

A factorial design approach using a 3^3 full factorial design composed of 3 variables which were set at 3-levels each was applied to maximize the experimental efficiency requiring a minimum of experiments. For this purpose three different variables and their influence on the physicochemical properties of the produced LN were analyzed. The design required a total of 11 experiments. The independent variables were the concentration of solid lipid S100, concentration of lecithin (Lipoid® S75) and the concentration of hydrophilic surfactant P188. The established dependent variables were the mean particle size (Z-Ave), polydispersity index (PI) and zeta potential (ZP). For each factor, the lower, medium and higher values of the lower, medium and upper levels were represented by a (−1), a (0) and a (+1) sign, respectively (Table 1). The data were analyzed using the STATISTICA 7.0 (Stafsoft, Inc.) software.

2.3. Lipid nanoparticles production

LN dispersions were prepared using a novel multiple emulsion (w/o/w) technique (García-Fuentes et al., 2003). Briefly, an inner w/o emulsion was initially prepared. A volume of ultra-purified water was added to the lipid phase (5 wt%) composed of glycerol, S100 and Lipoid® S75 at same temperature (5–10 °C above the melting point of the solid lipid Softisan® 100 ($T \approx 50$ °C) and homogenized 60 s with a sonication probe (6 mm diameter) by means of an Ultrasonic processor VCX500 (Sonics, Switzerland). A power output with amplitude of 40% was applied. A few milliliters of P188 solution was added and homogenized for additional 90 s. This pre-emulsion was poured in the total volume of a P188 cooled solution under magnetic stirring for 15 min to allow the formation of the LN. The obtained LN dispersions were used for subsequent studies. The general composition of LN dispersions is described in Table 2.

2.4. Physicochemical characterization

Physicochemical parameters such as Z-Ave, PI and ZP were analyzed by dynamic light scattering (DLS, Zetasizer Nano ZS, Malvern Instruments, Malvern, UK). All samples were diluted with ultra-purified water and analyzed in triplicate. For analysis of the ZP, ultra-purified water with conductivity adjusted to −50 μS/cm was used.

Table 2
Composition of SLN dispersions (wt/wt%).

Components	% (wt/wt)
Softisan® 100	5.0
Glycerol	37.5
Lipoid® S75	0.5
Lutrol® F68	1.0
Water add.	100