European Journal of Pharmaceutics and Biopharmaceutics 81 (2012) 448–452

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Note

siRNA LNCs – A novel platform of lipid nanocapsules for systemic siRNA administration

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ARTICLE INFO

Article history:
Received 24 November 2011
Accepted in revised form 13 February 2012
Available online 21 February 2012

Keywords:
Nanocarrier
Lipoplexes
Formulation
siRNA quantification
Cationic lipids
Systemic administration

ABSTRACT

Several siRNA (small interfering RNA) therapeutics are undergoing clinical trials for cancer, respiratory diseases or macular degeneration, but most are administrated locally. In order to overcome the different barriers to attain an efficient siRNA action after systemic administration, nanocarriers able to carry and protect siRNA are awaited. With this aim, we developed a new platform of siRNA lipid nanocapsules (LNCs) using different cationic lipids, combining the properties of LNCs (siRNA protection and targeting) and lipoplexes (efficient siRNA delivery into the cell). The formulation was revealed to contain different compartments. A siRNA quantification method based on UV spectroscopy was developed to locate and quantify siRNA in each compartment. All in all, these novel siRNA LNCs presented sizes of about 55 nm with a neutral surface charge and siRNA encapsulation efficiencies up to 65% representing appropriate characteristics for systemic administration.

1. Introduction

siRNA is rapidly broken down by nucleases and other blood components after intravenous administration. Moreover, between the administration and action site, different barriers have to be overcome. Therefore, different siRNA modifications and siRNA vectors have already been described in the literature, with a view to finding the "ideal" vector [1].

Lipid nanocapsules (LNCs), consisting of a lipid liquid core of triglycerides and a rigid shell of lecithin and polyethylene glycol, were developed in our laboratory [2]. The simple formulation process is based on phase inversions of an emulsion. These LNCs were recently modified to encapsulate DNA, complexed with cationic lipids, forming lipoplexes. These DNA LNCs were efficient for *in vitro* and *in vivo* transfection [3–5] in contrast to lipoplexes that were only efficient *in vitro*.

The association between LNCs and lipoplexes should combine LNC properties (nucleic acid protection, prolonged circulation time, possibility of active or passive targeting) with lipoplex properties (internalisation in cells, permitting nucleic acid action). With this in mind, the objective of this work was to develop siRNA LNCs allowing efficient systemic siRNA administration. In this study, various ratios of different cationic lipids were used to formulate siRNA LNCs, and the size, charge and payload characteristics of these novel promising nanocarriers were assessed.

2. Materials and methods

2.1. siRNA LNC formulation

Basic lipid nanocapsules (LNCs) were formulated, as described before [2], by mixing 20% w/w Labrafac WL 1349 (caprylic-capric acid triglycerides, Gatefossé SA, Saint-Priest, France), 1.5% w/w Lipoid S75-3 (Lipoid GmbH, Ludwigshafen, Germany), 17% w/w Solutol HS 15 (BASF, Ludwigshafen, Germany), 1.8% w/w NaCl (Prolabo, Fontenay-sous-Bois, France) and 59.8% w/w water (obtained from a Milli-Q system, Millipore, Paris, France) together under magnetic stirring. Three temperature cycles between 60 and 95 °C were performed to obtain phase inversions of the emulsion obtained after mixing all the components. Then, a rapid cooling and dilution with ice-cooled water (1:1.4) at the phase inversion temperature (PIT) led to LNC formation.

To obtain siRNA LNCs, the water in the last step was replaced by lipoplexes which were prepared by adding equal volumes of siRNA (here a model siRNA targeted against PCSK9, (sense sequence: GGAAGAUCAUAAUGGACAGdTdT) Eurogenetec, Seraing, Belgium) and liposomes in a defined charge ratio of cationic lipid charge

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0939-6411/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2012.02.010

versus anionic siRNA charge. NaCl was added during preparation to obtain a final concentration of 0.15 M.

For liposome preparation, a cationic lipid DOSP (dioleylamine-succinyl paromomycin) (synthesis previously described in [6]), bis(guanidinium)-tris(2-aminoethyl)amine-cholesterol (BGTC) (synthesis previously described in [7]) or DOTAP (1.2-dioleyl-3-trimethylammoniumpropane) (Avanti® Polar Lipids Inc., Alabaster, AL, USA), solubilised in chloroform, was weighted in the ratio 1/1 (M/M), 3/2 (M/M) or 1/1 (M/M), respectively, with the neutral lipid 1.2-dioleyl-sn-glycero-3-phosphoethanolamine (DOPE) (Avanti® Polar Lipids Inc., Alabaster, AL, USA) to obtain a final concentration of 20 mM of cationic lipid charge, considering the number of lipid charges per molecule (4 for DOSP, 2 for BGTC and 1 for DOTAP). After chloroform evaporation under vacuum, deionised water was added to hydrate the lipid film overnight at 4 °C which was sonicated the next day.

DOSP micelles were prepared in the same way without the addition of DOPE.

3. Characterisation

3.1. Size and zeta potential measurements

Size and zeta potential of siRNA LNCs were measured using a Malvern Zetasizer® (Nano Series ZS, Malvern Instruments SA, Worcestershire, UK) at 25 °C, in triplicate, after dilution in a ratio of 1:100 with deionised water.

3.2. Agarose gel electrophoresis

To verify siRNA encapsulation in LNC, Triton® X100 (Sigma, Saint-Quentin Fallavier, France) was added to destroy LNCs. Samples were mixed with OrangeBlue loading dye (Promega, Madison, WI, USA) before deposition on 1% agarose gel containing ethidium bromide (Sigma, Saint-Quentin Fallavier, France) and migration at 100 V for 30 min.

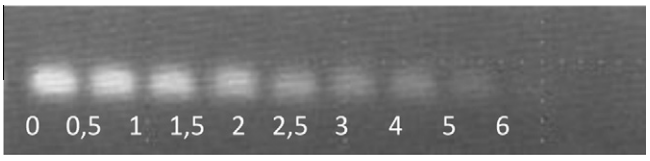
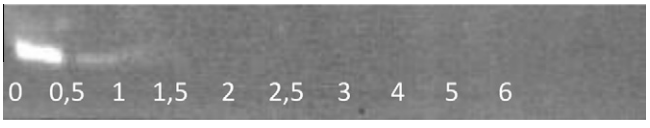
3.3. siRNA LNC purification

Five hundred microlitre of siRNA LNC formulation was deposited on a 1.5 × 40 cm Sepharose CL-4B column and were eluted with HEPES buffer (pH 7.4). Fractions of 500 µl or 1 ml were collected in glass tubes for further analysis; 100 µl of each fraction was used for turbidity measurements and analysed at 580 nm. PEG (polyethylene glycol) was quantified using 20 µl of each fraction, which was mixed with 5 µl KI/I_2 and 180 µl H_2O milli-Q before analysing at 492 nm using a Multiskan Ascent microplate reader (Thermo Fisher Scientific Cergy-Pontoise, France). Size and zeta potential measurements were taken as described above, and siRNA was evidenced using gel electrophoresis experiments.

3.4. siRNA quantification

One volume of the formulation was mixed with three volumes water (obtained from a Milli-Q-plus® system, Millipore, Paris, France), six volumes 1 M NaOH and two volumes chloroform, vortexed and immediately centrifuged for 15 min at 20,000 g and 4 °C. The aqueous phase, containing free siRNA and siRNA liberated from lipoplexes outside LNCs, was removed and analysed with a UV spectrophotometer (UVIKON 922, Kontron Instruments, Munich, Germany) at 260 nm. The volume removed for free siRNA quantification was replaced by ethanol. Then two volumes water and 10 volumes NaOH 1 M were added before vortexing and centrifuged a second time for 15 min at 20,000 g and 4 °C. The aqueous phase, containing the liberated siRNA from lipoplexes inside siRNA LNCs, was removed and analysed as previously mentioned at 260 nm. To analyse free and encapsulated siRNA quantity in siRNA LNCs, the same procedure was used using two volumes of formulation and replacing water by 1 M NaOH. The first aqueous phase contained free siRNA; the second aqueous phase contained siRNA encapsulated in siRNA LNCs. The siRNA quantity was calculated using a calibrating curve with different siRNA concentrations and compared to the total siRNA amount encapsulated in theory in siRNA LNCs. All samples were prepared in duplicate.

Table 1
Characteristics of siRNA lipoplexes used for siRNA LNC formulations.

Electrophoresis experiments	Lipid	CR	Mean diameter (nm)	Zone
	DOSP	2	1818	B
		2.5	1423	B
		3	1858	B
		4	1122	B
		5	605	C
	DOSP/DOPE	2	2229	B
		2.5	2026	B
		5	234	C
	BGTC/DOPE	2.5	1314	B
		3	770	B
		4	344	C
	DOTAP/DOPE	2.5	1110	B
		5	680	C