International Journal of Pharmaceutics 454 (2013) 756–764

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

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

Lipid nanocapsule functionalization by lipopeptides derived from human papillomavirus type-16 capsid for nucleic acid delivery into cancer cells

M. Weyland[a,b,1], A. Griveau[a,b,1], J. Bejaud[a,b], J-P. Benoit[a,b], P. Coursaget[c], E. Garcion[a,b,*]

[a] Inserm U1066, Micro et nanomédecines biomimétiques, F-49933 Angers, France
[b] LUNAM Université, F-49933 Angers, France
[c] Faculté des Sciences Pharmaceutiques, 31 Avenue Monge, F-37200 Tours, France

ARTICLE INFO

Article history:
Received 28 February 2013
Received in revised form 6 June 2013
Accepted 10 June 2013
Available online 14 June 2013

Keywords:
Papillomavirus derived peptide
Nanoparticles
DNA
siRNA
Vectorization
Cancer

ABSTRACT

Plasmid DNA (pDNA) and small interfering RNAs (siRNAs) are very useful tools for the treatment of cancer. However, pDNA and siRNAs efficacy is restricted by their negative charge and susceptibility to degradation by endonucleases that prevent them penetrating tissue and cellular barriers such as the plasma and endolysosomal membranes. Viral vectors have some advantages but their use is largely limited by their immunogenicity. On the other hand, synthetic nanoparticles have advantage of being relatively non-immunogenic but their ability to deliver nucleic acids remains less efficient than their viral counterparts. The present study is focussed on the development and evaluation of biomimetic lipid nanocapsules (LNCs) functionalized with a L1 papillomavirus type-16 capsid-derived lipopeptide on their surface, for transfection of U87MG glioma cells and Caco-2 colorectal adenocarcinoma cells with pDNA or siRNAs. Since the L1-peptide has been described as a nuclear localization signal able to complex with nucleic acids and bind to heparan sulfate on the cell surface, the structure and function of L1-peptide bound to LNCs (L1-LNCs) were investigated. Although L1-LNCs were shown to complex with both pDNA and siRNAs, the pDNA-L1-LNC complexes showed only weak transfection efficiency. In contrast, siRNA-L1-LNC complexes appeared as effective repressors of targeted messengers.

1. Introduction

Since cancer development relies on the acquired or inherited genetic and epigenetic activation of oncogenes and on the inactivation of tumour suppressor genes, substituting altered genes by healthy plasmid DNA (pDNA) copies may be used for therapy (Ortiz and Endy, 2012). Gene insertion into cancer cells using pDNA may also be useful to render them more sensitive to radiochemotherapy or to introduce discriminatory suicide genes (Zhang et al., 2008). pDNA-based therapy would also be beneficial for vaccination by generating adaptive immunity against tumour cells notably through MHC class I restricted cytolytic T-lymphocyte responses (Liu, 2011). As well as pDNA, small interfering RNAs (siRNAs), 20–25-nucleotide long double-stranded RNA (dsRNA) fragments, have demonstrated their capacity to mediate specific silencing of homologous genes (Elbashir et al., 2001; Fire et al., 1998; Wianny and Zernicka-Goetz, 2000). By interacting with the RNA-induced silencing complex (RISC) located in the cell cytoplasm, siRNAs lead to the degradation of complementary mRNA sequences and thus specifically down regulate the production of the encoded protein. The therapeutic potential of siRNAs has been investigated with some success, leading to clinical trials (Davis et al., 2010).

However, the use of pDNA or siRNAs for therapeutic purposes copies is hindered by efficacy, safety and delivery concerns that limit their clinical application. In order to have the intended effect these nucleic acids need to reach their subcellular targets: the nucleus for pDNA and the RISC complex in the cytoplasm for siRNAs. Hence, multiple tissue and cellular barriers need to be overcome, including intracellular uptake, largely prevented because of their negative charge and hydrophilicity (Lam et al., 2012), ability to escape endolysosomal maturation (El Ouahabi et al., 1997) and susceptibility to degradation by nucleases (Kawabata et al., 1995).

In this context, nanomedicine, represented by liposomes, polymeric nanospheres, lipid nanoparticles or nano-emulsions, may provide a solution. When encapsulated or complexed, the nucleic acid is no longer dependent only on its intrinsic properties but also on those of its carrier. Substantial benefits would be obtained

* Corresponding author at: Inserm U1066, IBS-CHU Angers, 4 rue Larrey, 49933 Angers cedex 9, France. Tel.: +33 2 44 68 85 43; fax: +33 2 44 68 85 46.
E-mail address: emmanuel.garcion@univ-angers.fr (E. Garcion).
[1] These authors contributed equally to this work.

0378-5173/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2013.06.013

in the crossing of biological barriers, the kinetics of nucleic acid release, bioavailability and efficacy in the target organs and cells, preventing side effects and permitting reduction of doses (Peer et al., 2007; Torchilin, 2009). However, although relatively non-toxic or non-immunogenic, non-viral vectors to deliver nucleic acids with only weak transfection efficacy (Perez-Martinez et al., 2011). In contrast, viral vectors are known for their high efficacy of transfection notably due to their well-adapted endolysosomal escape signals (Cho et al., 2003), but faces major limitations due to their immunogenicity and the risk of triggering serious sides effects (Hacein-Bey-Abina et al., 2008).

One possibility would be, therefore, to combined the advantages of each of these strategies by functionalizing nanoparticles with biomimetic peptides (Ma et al., 2012). Indeed, following the discovery of the first cell penetrating peptide derived from *Drosophila antennapedia homeodomain* (Derossi et al., 1994), they were investigated for their ability to deliver active molecules into cells (Poillot and De Waard, 2011). Furthermore, for potential therapeutic applications they were combined with nanoparticles (Torchilin, 2009). Biomimetic peptides may be used as active entities on their own or as targeting moieties capable to recognize specific biological components or receptors (Kim and Huang, 2012; Pearce et al., 2012; Ruoslahti, 2012; Su et al., 2012). In this respect, it was recently established that an L1 motif sequence (TSTTAKRKKRKLK) derived from papillomavirus type 16 capsid protein was sufficient to trigger heparan sulfate recognition, endocytosis and efficient gene transfer into target cells (Bousarghin et al., 2003a,b, 2004, 2009).

Thus, by focusing on lipid nanocapsules (LNCs) that have been confirmed as carriers for conventional anticancer drugs (Garcion et al., 2006; Lacoeuille et al., 2007; Weyland et al., 2011) and radiopharmaceutics (Vanpouille-Box et al., 2011), while also eliciting beneficial adjuvant effect such as inhibition of multidrug resistance (Garcion et al., 2006) or endolysosomal escape (Paillard et al., 2010; Roger et al., 2010), the aim of the present study was to develop LNCs combined with the L1-peptide and to investigate their aptitude for the delivery of pDNA and siRNAs to cancer cells.

2. Materials and methods

2.1. Preparation of control LNCs and Nile Red-loaded LNCs (NR-LNCs)

50 nm LNCs were prepared according to the process described elsewhere (Garcion et al., 2006), using a phase inversion process following the formation of an oil/water microemulsion containing an oily/fatty phase (caprylic-capric acid triglycerides: Labrafac® WL 1349; Gattefossé, Saint-Priest, France), a non-ionic hydrophilic surfactant (polyethylene glycol hydroxystearate: Solutol® HS15; Basf, Ludwigshafen, Germany) and a lipophilic surfactant (soybean lecithin containing at least 69% of phosphatidylcholine: Lipoïd® S75-3; Lipoïd GmbH, Ludwigshafen, Germany). Briefly, Solutol® HS15, Lipoid® S75-3, Labrafac® WL 1349, NaCl and water (846, 75, 1028, 89 and 2962 mg respectively) were mixed and heated under magnetic stirring to 90 °C. Then, three cycles of progressive heating and cooling between 90 °C and 60 °C were carried out, followed by an irreversible shock induced by dilution with 5 mL of cool deionized water added to the mixture at 80 °C. For the synthesis of fluorescent LNCs, the fluorescent compound Nile Red (NR; Sigma–Aldrich, Saint-Louis, USA) was used as previously described (Garcion et al., 2006). Briefly, NR was dissolved in acetone at 0.1% (w/w), and the 0.1% NR solution in acetone was incorporated in Labrafac® at 1:10 (w/w). Then, LNCs were prepared as described above with complete evaporation of the acetone during the process. Size exclusion and high-pressure liquid chromatography (HPLC) assays demonstrated a complete encapsulation and retention of NR within the LNCs (Garcion et al., 2006).

2.2. Method 1: Incorporation of DSPE-PEG2000-maleimide into the LNC shell and peptide conjugation

Firstly, 20 mg DSPE-PEG2000-maleimide (2941.605 g/mol, Avanti Polar Lipids, Alabaster, USA) powder diluted in HEPES buffer (0.1 M, pH 7.4), was added to 60 mg of LNCs suspension in order to obtain a final concentration of 20 mM. LNCs and DSPE-PEG2000-maleimide were incubated for 2 h at 60 °C. A Sepharose® CL4-B column (Sigma–Aldrich), equilibrated with HEPES buffer, was used to separate the functionalized LNCs from free DSPE-PEG2000-maleimide. The LNC concentration of the mixture was estimated by turbidity at 580 nm using a Multiskan® microplate spectrophotometer (Thermo Electron, Saint-Herblain, France). Secondly, 1.2 mg functionalized LNCs containing DSPE-PEG2000-maleimide were incubated with different amounts (between 0.6 μg and 6 μg of peptides) of L1 papillomavirus-derived peptide (L1; sequence: CTSTTAKRKKRKLK, 1313 g/mol; Millegen, Labège, France) or control peptide (CP; sequence: CLVEETSFIDAGAPS, 1280 g/mol; Millegen) overnight in HEPES buffer at room temperature under magnetic stirring. A Sephadex™ G25 Medium column (GE Healthcare Bio-Sciences AB, Sweden) equilibrated with HEPES buffer was used to remove unbound peptides. Turbidimetry measurement was performed for each fraction to detect the nanocapsules. Then, they were pooled and it is concentration was assessed by turbidimetric measurement at 580 nm. The amount of peptide grafted in the particles was determined by a micro Bradford colorimetric protein assay kit (microBCA, Perbio Science, Courtaboeuf, France).

2.3. Method 2: Lipopeptide monomer formation and post-insertion in LNC shell

A 1 mM DSPE-PEG2000-maleimide solution was prepared by dissolving 4.3 mg of DSPE-PEG2000-maleimide powder in 1.5 mL of HEPES buffer. To synthesize lipopeptides, DSPE-PEG2000-maleimide solution was incubated in the presence of different amounts of peptide (L1 or CP) overnight, at room temperature under magnetic stirring. The molar ratio of DSPE-PEG2000-maleimide/peptide was varied from 1/0.9 to 1/2.

100 μL of control LNCs at 200 mg/mL were incubated with 1 mL of lipopeptide solution for 4 h at 37 °C under magnetic stirring. A Sephadex™ G25 medium column equilibrated with HEPES buffer was used to separate the peptide-LNCs (L1- or CP-LNCs) from free DSPE-PEG2000-maleimide. To determine the peptide concentrations grafted onto the LNCs, a microBCA assay kit was used according to the manufacturer's instructions. The concentration of LNCs was evaluated by turbidimetric measurements at 580 nm using a Multiskan® microplate spectrophotometer.

2.4. Calculation of the number of peptides at the surface of LNCs following coupling by methods 1 and 2

Considering that all the excipients used in the formulation contribute to the formation of LNCs and the volume of these nanocapsules is mainly due to the Labrafac® (whose relative density is 0.94), it was possible to estimate the number of peptides (NP) on the surface of a single nanoparticle in each sample with the formula: $NP = ([peptide] \times N_A)/(V_{Labrafac®}/V_{LNC})$, where [peptide] is the amount of peptide as a molar concentration, N_A is the Avogadro number, $V_{Labrafac®}$ is the volume of Labrafac® in the sample and V_{LNC} is the volume of a single LNC calculated from the size measurements employing the volume of sphere formula, $4/3\pi R^3$.