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Research paper

Solid lipid nanoparticles can effectively bind DNA, streptavidin and biotinylated ligands

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Abstract

Cationic solid lipid nanoparticles (SLN) have recently been suggested for non-viral gene delivery, as these particles consist of well tolerated substances, can bind DNA directly via electrostatic interactions and mediate gene transfer in vitro. We here report the development of SLN complexes, which can be targeted to specific surface receptors. A formulation of SLN was prepared by the microemulsion technique comprising of stearylamine and the matrix lipid Compritol ATO 888 with a size of approximately 100 nm and a zeta-potential of +15. These SLN are able to condense DNA in complexes, which are very stable under physiological conditions, and they display low cytotoxicity in cell culture. In addition to binding of DNA, the SLN can simultaneously bind substantial amounts of streptavidin directly via electrostatic interactions. The SLN:DNA:streptavidin complexes are stable and are capable of binding biotinylated ligands, which can interact with surface receptors. This method allows for development of a fast and simple method of preparing a targeted non-viral gene therapy vector.

Keywords: Solid lipid nanoparticles; SLN; Cancer gene therapy; Targeted delivery; Streptavidin

1. Introduction

Several types of approaches have been attempted for development of drug delivery systems for genes including both viral and non-viral approaches. Apart from the many undesirable effects of most of these gene therapy vectors, such as immunogenic effects, toxicity, rapid clearance, trapping in specific organs etc., one of the major disadvantages of most formulations is that they confer no cellular specificity and have to be applied locally. For disseminated cancer diseases, such as many cancer forms, treatment must be administered systemically and therefore must be targeted to the cancer cells. Many efforts have been made for preparing targeted non-viral gene therapy vectors based on liposomes including cationic lipids (reviewed in [1]) or polyplexes, such as polyethyleneimine (PEI) (reviewed in [2]).

In the recent years, solid lipid nanoparticles (SLN) have been developed as potential carriers for a number of drugs (reviewed in [3,4]). SLN usually consists of physiologically well-tolerated ingredients already approved for pharmaceutical application in humans, can readily be produced in large scale, have good storage capabilities including freeze-drying, can be sterilised and show low cytotoxicity, when injected intravenously [3,5–7]. In addition, the advantage of SLN is that the charge of the particles can be modulated via the composition, thus allowing binding of oppositely charged molecules via electrostatic interactions. SLN can be produced in nano-scale size (100–200 nm), wherefore the particles are sufficiently small to traverse the microvascular system and prevent macrophage uptake and are therefore particularly suitable for systemic delivery.

There are only a few reports of the use of SLN for delivery of genes [8–11]. Olbrich et al. [8] prepared several SLN compositions by the high-pressure homogenisation method with positive ζ-potentials, but found only one composition, which could bind DNA and mediate expression of a reporter gene in cell culture. However, the transfection efficiency was extremely low compared to conventional polyplex transfection

Abbreviations BSA, bovine serum albumin; EGF, epidermal growth factor; EGFP, enhanced green fluorescent protein; PBS, phosphate buffered saline; PEI, polyethyleneimine; SLN, solid lipid nanoparticles.

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with poly-*L*-Lysine (P-*LL*) or PEI. This and other formulations were recently further tested by Tabatt et al. [9,11] and several formulations were demonstrated to have low cellular toxicity and bind significant amounts of DNA, but the transfection efficiencies remained very low compared to conventional agents. The transfection efficiency was significantly improved in vitro by pre-complexing the DNA with a dimer of the HIV-1 TAT-peptide, which contains a cell penetrating domain for improvement of cellular uptake and a nuclear localisation sequence for translocation of the DNA into the nucleus [10]. Although this SLN complex could not mediate substantial expression in vivo application, it may prove a potential method for administration of a therapeutic gene in a local environment, such as the pulmonary tract or for solid tumours.

The aim of the present study was to develop a SLN vector, which was able to complex with DNA, confer low or no transfection efficiency per se, but be able to be coupled to a ligand for targeted uptake via internalising receptors. We here report the production of an SLN formulation prepared by the microemulsion technique, which in addition to binding of significant amounts of DNA also could bind streptavidin in a stable complex. This SLN complex could bind directly to biotinylated ligands.

2. Materials and methods

2.1. Materials

Stearylamine, human recombinant EGF, bovine BSA, chloroquine and streptavidin were purchased from Sigma-Aldrich (Denmark), glyceryl dibehenate (Compritol® ATO 888) from Gattefossé (France), polysorbate 80 (Tween 80) from Bioworld (Germany), BODIPY 492/515 and LysoTracker Red® DND99 from Molecular Probes (Denmark). Cell culture reagents were purchased form Invitrogen (Denmark). The reporter plasmid pEGFP-N1 encoding enhanced green fluorescent protein was from Clontech (Denmark).

2.2. SLN production

The SLN were produced from a warm oil-in-water microemulsion as described in [12]. Briefly, the SLN formulation (final total lipid concentration 3.75 μg/μl) consist of the cationic lipid stearylamine (0.075% w/w), the uncharged lipid Compritol (0.3% w/w) and butanol (0.7% w/w) melted in an oil bath with gentle stirring at 75–80 °C. After completely melting of the lipids, 1 ml of pre-heated MilliQ water was added. The surfactant Tween 80 was added drop-wise until a clear microemulsion was obtained and additional 2 ml pre-warmed water is added. The warm microemulsion was suctioned into a 75 °C pre-warmed syringe and under constant pressure instantly sprayed into 40 ml of ice cold water with stirring for 30 min. To remove surfactant, aliquots of the SLN were dialysed against PBS (137.9 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2 (Invitrogen, Denmark) or water at 4 °C for 24–48 h using dialysis tubes of cellulose ester (Spectrum, Denmark) with a 300 KDa cut off and stored up to 1 month at 4 °C. The SLN were tested for toxicity on cell culture for each new batch (see below). For SLN labelled with the fluorescent dye BODIPY, this was added to the lipid phase during preparation of the microemulsion.

2.3. ζ-potential measurements

The ζ-potential measurements were performed using a Zeta Master (Malvern Instrument). Field strength in the measurement cell was 30 V/cm. All measurements were carried out in a degassed NaCl solution with an ionic strength of 0.001 M pre-incubated 10 min prior to ζ-potential measurement.

2.4. Size measurements

Size measurements were carried out using dynamic light scattering technique using a DynaPro (protein solution). The measurements were performed in MilliQ water. The laser sensitivity was set to 100%, baseline tolerance was 10% and the channels from 4 to 120 were included in the measurements. According to these settings, at least 10 fitting measurements were performed in order to get a representative result. The final result was calculated from the results obtained from the regularisation histogram.

2.5. Binding of DNA and agarose gel electrophoresis

Plasmid DNA was added to the dialysed SLN in water or PBS in the ratios indicated in the various experiments and incubated for 30 min at room temperature. For analysis of DNA binding by gel electrophoresis the samples were diluted in water, PBS or serum free medium, OPTIMEM (Invitrogen, Denmark). Analysis by agarose gel electrophoresis was performed at 2.5 V/cm in 0.8% agarose gels in TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA) containing μg/μl ethidium bromide. The total amount of DNA added per lane was 0.5 μg for all samples.

2.6. Biotinylation of ligands

EGF and BSA were labelled with biotin using the EZ-Link™-NHS-Biotin biotinylation kit from Pierce (Denmark) according to manufacturers instructions in phosphate buffer pH 6. BSA was biotinylated using 10 M excess of biotin and EGF using 5 and 10 molar excess of biotin. Biotinylation degree was determined by the HABA-Avidin-assay from Pierce. The proteins were labelled with three biotin molecules per molecule BSA and by one biotin per molecule EGF.

2.7. Labelling of DNA

Plasmid DNA was fluorescently labelled with TRITC (rhodamine) using the Mirus Label IT® kit (Mirus Corporation, Denmark) according to manufacturers instructions with the ethanol precipitation method for purification.