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Research paper

A biodistribution study of solid lipid-polyethyleneimine hybrid nanocarrier for cancer RNAi therapy

Hui Yi Xue, Ngoc Tran, Ho Lun Wong *

School of Pharmacy, Temple University, 3307 North Broad Street, Philadelphia, PA 19140, United States

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ABSTRACT

Solid lipid-polymer hybrid nanocarrier (LPN) was previously reported to achieve high siRNA transfection efficiency and induce sustained RNAi-based chemosensitizing effect at cellular level. In this study, our objectives were to evaluate the *in vivo* biodistribution of LPNs in a prostate cancer model and determine the factors that potentially affect tumor penetration by LPNs. The LPN formulation with the highest transfection efficiency (64%) and stability was selected for the study. Mice bearing tumors of PC-3M cells were treated with LPNs labeled with IR780 or AF647-siRNA. Near infrared imaging showed that LPNs achieved favorable *in vivo* biodistribution with high tumor/low organ ratios. LPN accumulation was also observed in liver metastatic tissue. Result of extravasation study confirmed that encapsulated siRNA molecules were able to escape into the tumor tissue at the extravascular area. When LPN levels in large (volume > 750 mm^3) and small (<500 mm^3) tumors were compared, no significant difference was observed. However, both docetaxel pretreatment (72 h before LPN) and concurrent docetaxel treatment significantly enhanced the tumor LPN levels by 3.9- and 3.1-fold, respectively (both $p < 0.01$). In conclusion, LPN is a promising carrier system to deliver RNAi therapy to solid malignancies that also receive chemotherapy.

1. Introduction

RNA interference (RNAi) has been widely studied for therapeutic purpose since its discovery [1]. The use of RNAi triggers such as small-interfering RNA (siRNA) for treatment of malignancies is particularly appealing considering the possibility to silence the expression of essentially any oncogenic or drug resistance gene product in a potent, reversible and customizable manner [2,3]. However, the development of RNAi therapeutics has encountered various issues during the bench-to-bedside translation process [4]. Only until recent years encouraging clinical progress with a few RNAi products was reported, and this has led to renewed interest in this class of therapeutics [4–6]. Reviews on the tortuous path of the development of RNAi therapeutics suggested that suboptimal RNA delivery remains to be one of the rate-limiting factors [6,7]. Hence, for development of any RNAi-based therapeutics, it is of foremost importance to adequately evaluate and optimize their delivery and biodistribution.

The delivery issue is especially critical in developing systemic RNAi treatment of solid tumors. For efficient tumor delivery, the RNAi therapeutics need to stay in circulation for sufficient time, achieve high vascular escape at the tumor site, penetrate and accumulate well in the tumor tissues, and transfect the cancer cells and escape into the cytoplasm efficiently [8,9]. Meanwhile, the accumulation of RNAi agents in the non-target organs should be kept as low as possible to minimize potential toxicity [10]. By delivering RNAi therapeutics in a well-designed nanocarrier, the passive (through enhanced permeation and retention effect) and active targeting properties (by surface decoration with cancer targeting moieties) of the carrier may help achieve significant tumor RNA drug levels in a relatively specific manner [7,11]. These advantages have been reported in the successful clinical development of stable nucleic acid lipid particles (known as SNALPs or sometimes LNPs). Significant accumulation of SNALPs in solid tumors was reported in several studies [12–14].

The success of SNALPs highlights the advantages of using lipids and phospholipids as the main ingredients to carry RNAi therapeutics. Nanosystems that include solid lipids, e.g., solid lipid

Abbreviations: DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; DSPE-PEG, 2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]; EGF, epidermal growth factor; LNP, lipid nanoparticle; LPN, lipid-polymer hybrid nanocarrier; PEI, polyethyleneimine; RNAi, RNA interference; siRNA, small-interfering RNA; SNALP, stable nucleic acid lipid particle.

* Corresponding author.
E-mail addresses: holunwong2011@yahoo.com, ho-lun.wong@temple.edu (H.L. Wong).

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nanoparticles and nanostructured lipid carriers, are particularly appealing for drug delivery. In addition to the high biocompatibility, high biodegradability, low immunogenicity and good interaction with cell membranes that are the hallmarks of lipid-based devices, the solid lipid components may also provide sustained, controlled drug release [11,15]. To expand on the application of the conventional solid lipid nanoparticles, our group has previously developed a new hybrid nanocarrier known as solid lipid–polymer hybrid nanocarriers (LPNs) for siRNA therapy of cancer [16,17]. Hybrid nanocarriers are highly versatile because of the possibility to combine the various advantages conferred by different class of materials [18,19]. In LPNs, the polymeric component, polyethylenimine (PEI), formed complex with siRNA and is incorporated in solid lipid components after hydrophobic modification [18]. PEI has been a frequently studied class of polycationic polymers for gene transfection because of their ability to protect nucleic acids, good interaction with cell surfaces and excellent endosomal escape effect [20]. In our previous studies, we have demonstrated that in LPNs, the lipid components not only reduced the toxicity of PEI by more than half, but they also prevented premature release of siRNA for days, reduced siRNA degradation by up to 21%, significantly enhanced the transfection efficiency of PEI, and helped to achieve sustained intracellular siRNA release [16,17,21]. The target gene product survivin was consequently knocked down by LPNs to less than 20% of the baseline level for at least a week. In short, the LPNs have demonstrated strong potential for sustained RNAi nanotherapy.

With the physicochemical properties and performance at cell level already demonstrated, our key objectives in the present study were to evaluate the LPN biodistribution in an animal tumor model and investigate the effects of tumor size and chemotherapy co-treatment, two factors that may likely affect the LPN accumulation in the solid tumor target. The tumor model used in this study was initiated with a highly metastatic human prostate cell line PC-3M, which is known for expression of epidermal growth factor (EGF) receptor [22,23]. Prior to the biodistribution study the EGF level on LPN was therefore first optimized to ensure that good cancer targeting and transfection performance were preserved at cell level, so we could focus on the *in vivo* delivery studies. The findings in this study not only are valuable for the clinical development of LPNs, but they may also provide the researchers with good information when designing other solid lipid-based or hybrid nanosystems for nucleic acid delivery to solid malignancies.

2. Materials and methods

2.1. Chemicals and reagents

Tripalmitin and triolein were purchased from TCI America Chemicals (Boston, MA). 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) and 2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG) were ordered from Avanti Polar Lipids (Alabaster, AL). 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[(polyethylene glycol)-3400] maleimide (DSPE-PEG-maleimide) was purchased from Nanocs (New York, NY, USA). Negative-siRNA (Non-targeting siControl #3, 5′-AUGUAUUGGCCUGUAUUAG-3′) was purchased from Dharmacon (Chicago, IL), and Rhodamine-siRNA (5′-UUCUCCGAACGUGUCACGUdTdT-3′, rhodamine conjugated) and AF647-siRNA (5′-UUCUCCGAACGUGUCACGUdTdT-3′, Alexa Fluor 647 conjugated) were purchased from Qiagen (Valencia, CA). Linear PEI (2500 Da) was purchased from Polysciences (Warrington, PA), dissolved in dichloromethane-methanol (4:1 v/v), filtered and lyophilized before use. PEI was modified by grafting hexadecyl groups to form hexadecylated PEI and purified using the method previously reported [16]. Other chemicals including human EGF and IR780 dye were bought from Sigma-Aldrich (St. Louis, MO). Water used for all RNA works was RNAase-free.

2.2. Cell cultures

PC-3M, the metastatic sub-line of human prostate epithelial PC3 cells, was a gift donated from Dr. Raymond Bergen at Northwestern University. The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 50,000 units penicillin G and 50,000 μg streptomycin at standard cell culture conditions (37 °C, humidified atmosphere of 5% CO_2 in air). Cells were passaged every 5–7 days and used for experiments from 10th to 25th passages.

2.3. EGF conjugation and nanocarrier preparation

DSPE-PEG-EGF was prepared with EGF and DSPE-PEG_{3400}-maleimide using standard thiolation protocol [24]. EGF in TEA buffer (50 mM triethanolamine, 150 mM NaCl, 1 mM EDTA, pH 8.0) was incubated with 2-iminothiolane at 5:1 M ratio at room temperature for 1 h, dialyzed (MWCO: 4 kDa, tube-O-dialyzer, G-Biosciences, St. Louis, MO) with excess MES buffer (50 mM MES, 2 mM EDTA, pH 6.0) to desalt, then added to DSPE-PEG-maleimide in HEPES buffer (20 mM HEPES, 150 mM NaCl, pH 7.4) and incubated at room temperature for 2 h. Free EGF was removed by dialyzing (MWCO: 8 kDa) the product in double distilled water three times. DSPE-PEG-EGF was lyophilized for LPN preparation.

Non-targeting LPNs were prepared using emulsification-solvent evaporation technique as previously reported [16,17]. 15 nmole siRNA was precomplexed with 0.7 mg hexadecylated PEI in a dichloromethane solution containing 2 mg triolein. The mixture was added to the mixture of lipids including cetyl palmitate, tripalmitin, triolein, DSPC and DSPE-PEG2000 at molar ratio of 40:15:18:15:12 in dichloromethane. For LPN with EGF decoration, same molar amount of DSPE-PEG2000 was substituted with DSPE-PEG-EGF. Nanocarriers were formed by sonicating the mixture in 3 ml of 5% dextrose solution (3 cycles × 1 min, 40 kHz, 120 V, Bransonic 3510, Danbury, CT) followed by magnetic stirring at 1200 rpm in vacuum at room temperature.

For *in vivo* biodistribution study, LPNs (loading siControl as a dummy RNA) were prepared with inclusion of a lipophilic near-infrared dye IR780 (at ratio of 4.5 nmole dye per nmole siRNA). To rule out premature release of IR780 from the nanoparticles, 10 mg LPN containing IR780 was dialyzed in 50 ml of cell culture medium at 37 °C for 48 h. Spectrophotometric analysis of the medium showed less than 4% dye was released within this period, indicating that the dye was well retained in LPNs, and was useful for imaging and monitoring of the nanocarrier distribution.

2.4. Size, zeta potentials and serum stability of LPNs

Photon correlation spectroscopy was used to evaluate nanoparticle size and zeta potential (Malvern Zetasizer NanoZS90, Worcestershire, UK) of LPNs with different EGF densities. For each measurement, 50 μg (for size) or 100 μg (for zeta potential) sample of nanocarriers was dispersed in 2 ml distilled water and fifteen successive cycles were run at 25 °C. A minimum of three samples were measured. Data based on the distribution by intensity were reported. To evaluate whether samples of LPN could remain well dispersed in the experimental conditions, suspensions of nanocarriers were incubated in phosphate buffered saline (pH7) supplemented with 10% FBS at 37 °C and stirred at 100 rpm for