Journal of Controlled Release 203 (2015) 140–149

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Selective targeting of alveolar type II respiratory epithelial cells by anti-surfactant protein-C antibody-conjugated lipoplexes

Yun Wu [a,b,*], Junyu Ma [b], Parker S. Woods [c], Nicholas M. Chesarino [d], Chang Liu [a], L. James Lee [b,e], Serge P. Nana-Sinkam [f], Ian C. Davis [c,**]

[a] Dept. of Biomedical Engineering, State University of New York at Buffalo, Bonner Hall, Buffalo, NY 14260, USA
[b] Nanoscale Science and Engineering Center for Affordable Nanoengineering of Polymeric Biomedical Devices, The Ohio State University, Smith Laboratory, 174W. 18th Ave., Columbus, OH 43210, USA
[c] Dept. of Veterinary Biosciences, The Ohio State University, Goss Laboratories, 1925 Coffey Road, Columbus, OH 43210, USA
[d] Dept. of Microbial Infection & Immunity, The Ohio State University, Biomedical Research Tower, 460W. 12th Ave., Columbus, OH 43210, USA
[e] William G. Lowrie Dept. of Chemical and Biomolecular Engineering, The Ohio State University, Koffolt Laboratories, 140W. 19th Ave., Columbus, OH 43210, USA
[f] Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, The Ohio State University, Davis Heart and Lung Research Institute, 473W. 12th Ave., Columbus, OH 43210, USA

ARTICLE INFO

Article history:
Received 14 November 2014
Received in revised form 30 January 2015
Accepted 12 February 2015
Available online 14 February 2015

Keywords:
Lipoplex
Alveolar type II cell
Surfactant protein-C
MicroRNA
Targeted delivery

ABSTRACT

Alveolar type II (ATII) respiratory epithelial cells are essential to normal lung function. They may be also central to the pathogenesis of diseases such as acute lung injury, pulmonary fibrosis, and pulmonary adenocarcinoma. Hence, ATII cells are important therapeutic targets. However, effective ATII cell-specific drug delivery in vivo requires carriers of an appropriate size, which can cross the hydrophobic alveolar surfactant film and polar aqueous layer overlying ATII cells, and be taken up without inducing ATII cell dysfunction, pulmonary inflammation, lung damage, or excessive systemic spread and side-effects. We have developed lipoplexes as a versatile nanoparticle carrier system for drug/RNA delivery. To optimize their pulmonary localization and ATII cell specificity, lipoplexes were conjugated to an antibody directed against the ATII cell-specific antigen surfactant protein-C (SP-C) then administered to C57BL/6 mice via the nares. Intranasally-administered, anti-SP-C-conjugated lipoplexes targeted mouse ATII cells with >70% specificity in vivo, were retained within ATII cells for at least 48 h, and did not accumulate at significant levels in other lung cell types or viscera. 48 h after treatment with anti-SP-C-conjugated lipoplexes containing the test microRNA miR-486, expression of mature miR-486 was approximately 4-fold higher in ATII cells than whole lung by qRT-PCR, and was undetectable in other viscera. Lipoplexes induced no weight loss, hypoxemia, lung dysfunction, pulmonary edema, or pulmonary inflammation over a 6-day period. These findings indicate that ATII cell-targeted lipoplexes exhibit all the desired characteristics of an effective drug delivery system for the treatment of pulmonary diseases that result primarily from ATII cell dysfunction.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

The primary physiologic function of the lungs is gas exchange, which occurs in the terminal bronchioles and alveoli of the distal lung. The bronchoalveolar epithelium is composed of a single layer of epithelial cells covered with a thin layer of aqueous fluid and an overlying film of surfactant [1]. Thin, squamous alveolar type I cells comprise only ~40% of the cells in the alveolus, but account for more than 90% of the surface area of the alveolar epithelium [2]. The remaining 5–10% is made up of small (~10 μm) cuboidal alveolar type II (ATII) cells [3]. ATII cells synthesize, secrete, and recycle pulmonary surfactant lipids and proteins, which contribute to the maintenance of low intra-alveolar surface tension and thereby facilitate ventilation [4]. ATII cells also regulate the depth of the alveolar lining fluid layer by active ion transport, participate in lung inflammatory responses, and serve as progenitors for alveolar type I cells [5–7]. Hence, ATII cells are essential to normal lung function. Importantly, they may be also central to the pathogenesis of multiple acute and chronic, potentially lethal lung diseases, including neonatal respiratory distress syndrome, acute lung injury, pulmonary fibrosis, pulmonary adenocarcinoma, and severe influenza, for which current treatment options are limited [8–12]. As such, ATII cells are important therapeutic targets.

There are several limitations to current methods for targeted delivery of nanoparticle therapeutics for in vivo applications, such as limited stability in serum, rapid blood clearance, poor cellular uptake, and off-target effects. To overcome these limitations, our group has developed

* Correspondence to: Y. Wu, Dept. of Biomedical Engineering, State University of New York at Buffalo, 332 Bonner Hall, Buffalo, NY 14260 USA.
** Correspondence to: I.C. Davis, Dept. of Veterinary Biosciences, College of Veterinary Medicine, 331 Goss Labs, 1925 Coffey Road, Columbus, OH 43210, USA.
E-mail addresses: ywu32@buffalo.edu (Y. Wu), junyuma@hotmail.com (J. Ma), Parker.Woods@osumc.edu (P.S. Woods), chesarino.1@buckeyemail.osu.edu (N.M. Chesarino), cliu36@buffalo.edu (C. Liu), lee.31@osu.edu (L.J. Lee), Patrick.Nana-Sinkam@osumc.edu (S.P. Nana-Sinkam), davis.2448@osu.edu (I.C. Davis).

http://dx.doi.org/10.1016/j.jconrel.2015.02.016
0168-3659/© 2015 Elsevier B.V. All rights reserved.

lipoplexes (LPs) as carrier systems for drug/nucleic acid delivery [13–16]. Because hydrophobic therapeutics can be incorporated in the lipid bilayers and hydrophilic therapeutics can be encapsulated in the liquid core of LPs, they are highly versatile. In previous studies, we showed that cationic LPs administered to mice by the intravenous route could target the lungs and be retained therein for at least 48 h without inducing obvious lung toxicity [13]. However, in addition to lung tissue, we found significant accumulation of LPs in the liver and kidneys of treated mice. The aim of the current study was therefore to develop a universal delivery platform that can specifically deliver drugs/nucleic acids to ATII cells, without off-target deposition in other cell types in the lung and other organs. To achieve this objective, we directly administered LPs to the mouse lung via the nares. We also determined the impact of conjugating LPs to a monoclonal antibody directed against the ATII cell-specific antigen surfactant protein C (SP-C) on targeting of a microRNA (miR) to that specific lung cell type. Synthetic miR-486 was selected as a model drug for LP delivery because miR-486 is one of the most down-regulated miRs in lung cancer [17–19]. Additionally, because the delivered amount of miR-486 can be quantitatively measured by qRT-PCR, drug delivery efficiency could be easily and accurately quantified.

2. Material and methods

2.1. Materials

1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Linoleic acid, polyethyleneimine (PEI, MW ~ 2000) and ethanol were purchased from Sigma-Aldrich (St Louis, MO, USA). D-α-tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS) was purchased from Eastman (Kingsport, TN, USA). Cy5 dye-labeled oligodeoxynucleotide (5′-Cy5-TCT-CCC-AGC-GTG-CGC-CAT-3′ [Cy5-ODN]) was custom synthesized by Alpha DNA, Inc. (Montreal, Canada). Rabbit polyclonal IgG anti-SP-C antibody FL-197 was purchased from Santa Cruz Biotechnology, Inc. (sc-13979; Dallas, TX, USA). MirVana™ miR mimic hsa-miR-486-5p (miR-486; UCC-UGU-ACU-GAG-CUG-CCC-CGA-G) and scrambled miR mimic Negative Control #1 (miR-SCR) were purchased from Life Technologies, Inc. (Grand Island, NY, USA).

2.2. Preparation of lipoplexes containing nucleic acids

Empty liposomes were first generated by injecting a lipid mixture in ethanol (DOPE:linoleic acid:TPGS at 50:48:2 molar ratio) into 20 mM HEPES buffer (pH = 7.4) to achieve 10% ethanol and 90% aqueous in the final mixture. An equal volume of 0.516 mg/ml PEI solution was added to 0.4 mg/ml nucleic acid solution (ODN^{Cy5}, miR-486, or miR-SCR) resulting in an N:P ratio (the ratio of moles of the amine group of PEI to moles of the phosphate groups of nucleic acid) of 10. The PEI/nucleic acid mixture was then sonicated for 5 min and incubated at room temperature for 10 min. LPs containing nucleic acids (LP-ODN^{Cy5}, LP-miR-486, or LP-miR-SCR) were prepared by adding the PEI/nucleic acid mixture to empty liposomes at a lipid:nucleic acid mass ratio of 10. The mixture was sonicated for 5 min and incubated at room temperature for 15 min.

2.3. Incorporation of anti-SP-C antibody onto lipoplexes

Anti-SP-C antibody was incorporated onto LPs by a post-insertion method, as previously described [20]. Briefly, anti-SP-C antibody was thiolated at its N-terminus with 2-iminothiolane (Traut's reagent) in PBS (pH = 8.0) and purified by gel filtration on a PD-10 column. The thiolated anti-SP-C antibody (anti-SP-C-SH) was then reacted with micelles of Mal–PEG–DSPE at a protein-to-lipid molar ratio of 1:10 for 3 h at room temperature in PBS (pH = 6.5) to obtain anti-SP-C–PEG–DSPE, which was then post-inserted onto LPs by co-incubation at 37 °C for 1 h. The molar ratio of lipids and anti-SP-C antibody was 2000:1. SP-C targeted LPs (LP-$ODN^{Cy5/ANTI\text{-}SP\text{-}C}$, LP-miR-$486^{ANTI\text{-}SP\text{-}C}$, or LP-miR-$SCR^{ANTI\text{-}SP\text{-}C}$) were concentrated using Amicon Ultra-15 Centrifugal Filter Units (UFC900308; Millipore, Billerica, MA, USA) so that preparations for *in vivo* applications contained miRs at a concentration of 0.7 mg/ml. Finally, absence of endotoxin contamination of LP preparations was confirmed by a standard *Limulus* amebocyte assay.

2.4. Lipoplex particle size and surface charge measurement

LP size distributions were measured by dynamic light scattering (BI 200SM; Brookhaven Instruments Corp., Holtsville, NY, USA). The wavelength of the laser was 632.8 nm, and the detection angle was 90°. The size distributions of three batches of LPs prepared independently were measured at 20 °C. LP surface charges were measured using a ZetaPALS zeta potential analyzer (Brookhaven Instruments). Three batches of independently prepared LPs were diluted in 20 mM HEPES buffer. Three measurements, each consisting of 5 runs, were performed at 20 °C. The Smoluchowski model was used to calculate the zeta potential.

2.5. Uptake of lipoplexes by A549 cells

A549 human lung adenocarcinoma cell uptake of LPs was evaluated by flow cytometry. 2×10^5 viable A549 cells/well were seeded in 6-well plates and incubated overnight in 2 ml of RPMI 1640 media supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA). The culture medium was then replaced with serum-free RPMI 1640. LP-ODN^{Cy5} and LP-$ODN^{Cy5/ANTI\text{-}SP\text{-}C}$ were added to the cells at an ODN^{Cy5} concentration of 1 μM. 4 h post-treatment, cells were detached from culture plates using 0.25% trypsin, washed with PBS twice, fixed using 4% paraformaldehyde, and subjected to analysis on a BD LSR Fortessa flow cytometer (Becton Dickinson, San Jose, CA, USA). Cy5 fluorescence was measured in the APC channel. 10,000 events were collected for each sample.

2.6. Animals

Pathogen-free, 8 week-old female C57BL/6AnNCr mice (*Mus musculus*) were purchased from the National Cancer Institute (Frederick, MD, USA). C57BL/6-congenic SP-C^{GFP} mice [21,22] (generously provided by Dr. Jo Rae Wight, Duke University, NC, USA) were bred in-house and used at 8 weeks of age. All mice were maintained in sterile caging and provided with food and water ad libitum. All animal procedures were approved by The Ohio State University Institutional Animal Care and Use Committee. Care and handling of all animals was in accordance with the NRC/NIH Guide for the Care and Use of Laboratory Animals.

2.7. Lipoplex administration

Mice were anesthetized by i.p. injection of ketamine (8.7 mg/kg)/xylazine (1.3 mg/kg). To facilitate pulse oximetry, the fur over the neck was removed by application of Nair (Church & Dwight, Ewing, NJ, USA) for 2 min. Mice were held vertically by the scruff and LPs (1.5 mg/kg, suspended in 50 μl saline) were administered dropwise to both nostrils. Animals were placed on a heat pad in left lateral recumbency then returned to their cages upon recovery. Treated mice were weighed every other day. Data for each experimental animal group were derived from a minimum of two independent experiments.

2.8. Whole organ imaging

Immediately prior to imaging, C57BL/6 mice were euthanized by i.p. injection of ketamine (87 mg/kg)/xylazine (13 mg/kg). The lungs, heart, spleen, liver, and both kidneys were removed by careful dissection and fixed in 10% formalin. Organ Cy5 fluorescence was detected using the

Download English Version:

https://daneshyari.com/en/article/7863658

Download Persian Version:

https://daneshyari.com/article/7863658

Daneshyari.com