Journal of Controlled Release 208 (2015) 121–129

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Cationic liposome–hyaluronic acid hybrid nanoparticles for intranasal vaccination with subunit antigens

Yuchen Fan [a,b,1], Preety Sahdev [a,b,1], Lukasz J. Ochyl [a,b], Jonathan J. Akerberg [a,b], James J. Moon [a,b,c,*]

[a] Department of Pharmaceutical Sciences, University of Michigan, Ann Arbor, MI 48109, USA
[b] Biointerfaces Institute, University of Michigan, Ann Arbor, MI 48109, USA
[c] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, USA

ARTICLE INFO

Article history:
Received 16 December 2014
Received in revised form 5 April 2015
Accepted 9 April 2015
Available online 11 April 2015

Keywords:
Liposome
Nanoparticle
Subunit antigen
Intranasal vaccine
Yersinia pestis

ABSTRACT

Here we report the development of a new cationic liposome–hyaluronic acid (HA) hybrid nanoparticle (NP) system and present our characterization of these NPs as an intranasal vaccine platform using a model antigen and F1-V, a candidate recombinant antigen for *Yersinia pestis*, the causative agent of plague. Incubation of cationic liposomes composed of DOTAP and DOPE with anionic HA biopolymer led to efficient ionic complexation and formation of homogenous liposome–polymer hybrid NPs, as evidenced by fluorescence resonance energy transfer, dynamic light scattering, and nanoparticle tracking analyses. Incorporation of cationic liposomes with thiolated HA allowed for facile surface decoration of NPs with thiol-PEG, resulting in the formation of DOTAP/HA core-PEG shell nanostructures. These NPs, termed DOTAP–HA NPs, exhibited improved colloidal stability and prolonged antigen release. In addition, cytotoxicity associated with DOTAP liposomes (LC_{50} ~ 0.2 mg/ml) was significantly reduced by at least 20-fold with DOTAP–HA NPs (LC_{50} > 4 mg/ml), as measured with bone marrow derived dendritic cells (BMDCs). Furthermore, NPs co-loaded with ovalbumin (OVA) and a molecular adjuvant, monophosphoryl lipid A (MPLA) promoted BMDC maturation and upregulation of co-stimulatory markers, including CD40, CD86, and MHC-II, and C57BL/6 mice vaccinated with NPs via intranasal route generated robust OVA-specific $CD8^+$ T cell and antibody responses. Importantly, intranasal vaccination with NPs co-loaded with F1-V and MPLA induced potent humoral immune responses with 11-, 23-, and 15-fold increases in F1-V-specific total IgG, IgG_1, and IgG_{2c} titers in immune sera by day 77, respectively, and induced balanced Th1/Th2 humoral immune responses, whereas mice immunized with the equivalent doses of soluble F1-V vaccine failed to achieve sero-conversion. Overall, these results suggest that liposome–polymer hybrid NPs may serve as a promising vaccine delivery platform for intranasal vaccination against *Y. pestis* and other infectious pathogens.

1. Introduction

Synthetic nanoparticles (NPs) are promising delivery systems for subunit vaccines composed of peptides, recombinant proteins, or DNA [1–3]. Advantages of particulate vaccines include efficient encapsulation of antigens, shielding of antigens from rapid enzymatic degradation, and ability to co-deliver antigens with molecular adjuvants to antigen-presenting cells (APCs), thus promoting cellular and humoral immune responses [3]. Among particulate vaccine delivery systems, liposomes of various lipid compositions have been widely investigated as potential vaccine carriers. In particular, cationic liposomes composed of 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) have been extensively studied as they can readily form nano-complexes with anionic peptides, proteins, and plasmid DNA encoding for antigens and generate T and B cell immune responses in vivo [4–8]. Despite significant advances made in this field, there are still several major challenges remaining for liposomal vaccines, including cytotoxicity of cationic liposomes that can negatively impact immune responses at high concentrations as well as their suboptimal in vivo stability for delivery of biomacromolecules [6–10]. We previously addressed some of these issues by developing a new lipid-based NP system formed by divalent cation-induced liposomal fusion into multilamellar vesicles and subsequent cross-linking of apposing lipid layers via maleimide–thiol reaction [11]. The resulting NPs released cargo protein in a stable manner and elicited robust humoral and cellular immune responses [11–13]. As an alternative approach to producing stable vaccine delivery systems, here we aimed to synthesize lipid–biopolymer hybrid NPs by exploiting ionic charge interactions between liposomes and hyaluronic acid (HA), which is a biodegradable polymer that has been shown to form complexes with liposomes [14] and investigated as a vaccine delivery agent [15–17]. Specifically, we utilized ionic complexation between cationic DOTAP-based liposomes and anionic HA-based biopolymers to form DOTAP–HA hybrid NPs, which were then surface-decorated with poly(ethylene glycol) (PEG), resulting in the

* Corresponding author at: 2800 Plymouth Road NCRC, Ann Arbor, MI 48109, USA.
E-mail address: moonjj@umich.edu (J.J. Moon).
[1] The authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jconrel.2015.04.010
0168-3659/

formation of DOTAP/HA core-PEG shell NPs. We report here that these NPs may serve as a promising vaccine delivery platform for intranasal vaccination.

Yersinia pestis, a causative agent of pneumonic plague, is a Category A bioterrorism bacterial agent that can be easily transmitted through pulmonary inhalation, potentially causing a death rate near 100% within a week of infection [18]. However, there are currently no available vaccine products against pneumonic plague. Therefore, it is of high priority to develop a protective plague vaccine. For vaccination against *Y. pestis*, intranasal route of immunization is attractive due to ease of vaccine administration and rapid deployment in the time of imminent biological threat. In addition, nasal cavity is characterized by highly permeable nasal epithelium for absorption of biomolecules and high frequency of immune cells within nasal-associated lymphoid tissues [19]. Thus, nasal vaccination against *Y. pestis* may drive induction of local mucosal immune responses in the airway to prevent initial pneumonic infection while simultaneously eliciting systemic immune responses to inhibit transmission of bacterial infection. In particular, F1-V, a recombinant fusion protein of fraction 1 pilus and LcrV antigen from *Y. pestis*, has been demonstrated to be a promising candidate for plague vaccine in a number of previous studies [18,20]. In addition, F1-V in combination with various types of adjuvants [21] or nanocarriers [22,23] has been shown to promote prophylactic humoral immune responses against *Y. pestis*.

In this study, we report the development of a new liposome–polymer hybrid NP system and our initial characterization of these NPs as an intranasal vaccine platform using a model antigen as well as F1-V. We show that DOTAP liposomes can be readily incorporated with thiolated HA (HA-SH) by promoting ionic complexation between DOTAP and HA-SH. The resulting DOTAP–HA NPs were further stabilized by reacting the HA-SH layer on the outer shell with thiolated PEG (PEG-SH), generating stable DOTAP/HA core-PEG shell NPs (Fig. 1). Importantly, cytotoxicity of DOTAP liposomes in BMDCs (LC_{50} ~ 0.2 mg/ml) was significantly reduced by at least 20-fold (LD_{50} > 4 mg/ml) for DOTAP–HA NPs. In addition, toll-like receptor (TLR) 4 agonist, MPLA [24], was chosen as a molecular adjuvant for both the model antigen OVA and F1-V. DOTAP–HA hybrid NPs co-loaded with antigens and MPLA promoted maturation of BMDCs in vitro and effectively stimulated antigen-specific cellular and humoral immune responses in vivo after intranasal vaccination, suggesting their potency as a promising nasal vaccine platform against infectious pathogens.

2. Materials and methods

2.1. Reagents

Lipids including 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), nitrobenzoxadiazole (NBD)-labeled DOPE (DOPE-NBD), rhodamine (Rhod)-labeled DOPE (DOPE-Rhod), and MPLA were all purchased form Avanti Polar Lipids (Alabaster, AL). Sodium hyaluronate (HA) and 2 kDa PEG-SH were from Lifecore Biomedical (Chaska, MN) and Laysan Bio (Arab, AL), respectively. L-cysteine, N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) and chloramine T were obtained from Sigma-Aldrich (St. Louis, MO). Ovalbumin (OVA) and F1-V were obtained from Worthington (Lakewood, NJ) and NIH BEI Resources (Manassas, VA), respectively. RPMI 1640 media, fetal bovine serum (FBS), penicillin–streptomycin, β-mercaptoethanol, ACK lysis buffer and Texas Red N-hydroxysuccinimide ester were from Life Technologies (Grand Island, NY). Granulocyte macrophage colony stimulating factor (GM-CSF) was the product of PeproTech (Rocky Hill, NJ). Rat anti-mouse CD16/32, CD86-PE, CD40-APC, and MHC Class II-FITC were from eBioscience (San Diego, CA). Rat anti-mouse CD8-APC, hamster anti-mouse CD11c-PE-

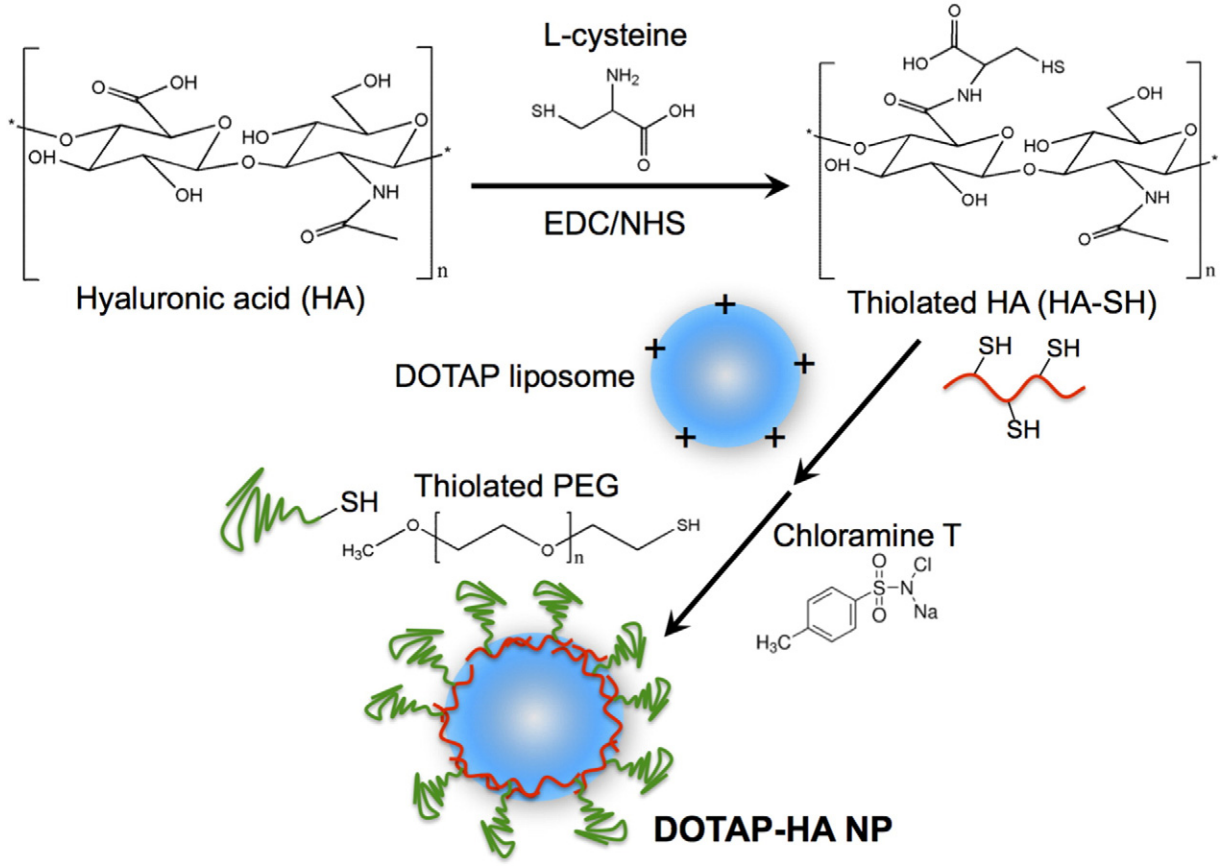

Fig. 1. Schematic illustration of thiolation of hyaluronic acid and formation of lipid–polymer hybrid nanoparticles.