Biomaterials 106 (2016) 228–239

Contents lists available at ScienceDirect

Biomaterials

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

The next-generation nicotine vaccine: a novel and potent hybrid nanoparticle-based nicotine vaccine

Yun Hu [a], Daniel Smith [a], Evan Frazier [a], Reece Hoerle [a], Marion Ehrich [b], Chenming Zhang [a, *]

[a] Department of Biological Systems Engineering, Virginia Tech, Blacksburg, VA 24061, USA
[b] Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA 24061, USA

ARTICLE INFO

Article history:
Received 10 June 2016
Received in revised form
15 August 2016
Accepted 17 August 2016
Available online 18 August 2016

Keywords:
Nicotine vaccine
Hybrid nanoparticle
Antibody
PLGA
KLH
Dendritic cell

ABSTRACT

Owing to the urgent need for more effective treatment against nicotine addiction, a hybrid nanoparticle-based nicotine vaccine (NanoNiccine) was developed in this study. NanoNiccine was composed of a poly(lactide-co-glycolide) acid (PLGA) core, keyhole limpet hemocyanin (KLH) as an adjuvant protein enclosed within the PLGA core, a lipid layer, and nicotine haptens conjugated to the outer surface of the lipid layer. In contrast to the traditional nicotine vaccine, NanoNiccine is not a nicotine-protein conjugate vaccine. Instead, the nicotine hapten and protein are separately located in the nanostructure to minimize antibody production towards KLH. The cellular uptake study demonstrated that NanoNiccine was ideal for internalization and processing by dendritic cells (DCs). Mice immunized with NanoNiccine produced much lower IgG level against KLH as compared to that immunized with the traditional nicotine-KLH (Nic-KLH) vaccine. In addition, NanoNiccine achieved up to a 400% higher titer of anti-nicotine IgG than the positive control, Nic-KLH. Additionally, the Th1/Th2 index of NanoNiccine suggested that the immune response induced by NanoNiccine was antibody response dominant. Furthermore, NanoNiccine was found to be safe in mice.

1. Introduction

Tobacco use continues to be the leading cause of preventable death worldwide, resulting in more than 6 million deaths and immeasurable economic loss each year [1]. It has been widely recognized that nicotine is the major component that is responsible for tobacco addiction [2]. Although, conventional pharmacotherapies [3] including nicotine replacement therapy, varenicline, and bupropion prove to be effective in treating nicotine addiction, the overall abstinence rate is highly limited and these therapies are more or less accompanied with adverse effects [4–6]. Therefore, there is an urgent need for a more effective and safer treatment method for nicotine addiction. In recent years, nicotine vaccines, which can induce the production of nicotine-specific antibodies and prevent nicotine entry into the brain, have exhibited great potential as a new-generation therapy to help people quit smoking [7]. Nicotine is a small compound and cannot induce immune response on its own; and thus it has to be associated with bigger molecules, such as proteins, for it to be immunogenic [8]. Following the above rationale, traditional nicotine vaccines share a common trait, in that nicotine haptens are covalently conjugated to proteins [9]. These vaccines prove to be effective in producing nicotine specific antibodies, and some of them have even advanced into clinical trials [10,11]. However, such a nicotine-protein conjugate design has some drawbacks, which may limit the treatment efficacy of the resulting vaccines. Firstly, antigen presenting cells (APCs), such as dendritic cell (DC), macrophage, and B cell, prefer to capture and internalize particulate antigens [12], including virus, bacteria, and nanoparticles, instead of soluble protein antigens; secondly, if not impossible, nicotine-protein conjugate vaccines can hardly co-deliver antigens and adjuvant molecules to target immune cells, in contrast, nanoparticles-based vaccine can easily achieve such a task [13]; and lastly, carrier proteins themselves are immunogenic, which may result in wastage of the nicotine-protein conjugate vaccine for eliciting antibodies against the protein rather than nicotine.

In order to overcome the above shortcomings of the traditional nicotine-protein conjugate vaccines, in this study, we designed a

* Corresponding author.
E-mail address: chzhang2@vt.edu (C. Zhang).

http://dx.doi.org/10.1016/j.biomaterials.2016.08.028
0142-9612/

novel lipid-PLGA hybrid nanoparticle-based nicotine vaccine (NanoNiccine). The major components of this vaccine are a PLGA core, a lipid surface layer, keyhole limpet hemocyanin (KLH) in the core, monophosphoryl lipid A (MPLA) as a molecular adjuvant in the lipid layer, and nicotine haptens covalently linked to the outer surface of the lipid layer. Different from the traditional nicotine-protein conjugate vaccine [14–16], KLH in the PLGA core of NanoNiccine solely served as a supplier of T cell antigens, instead of a carrier protein. This may reduce the possibility of generating antibodies against KLH. Another advantage of this design is that molecular adjuvants, such as MPLA [17], and CpG oligodeoxynucleotides (CpG ODNs) [18] can be co-delivered with antigens to immune cells, which may increase the magnitude of immune response. The immunogenicity of NanoNiccine and the traditional nicotine vaccine using KLH as a carrier protein (i.e. positive control) was studied in mice. The results showed that NanoNiccine generated a much higher titer of antibodies against nicotine than the traditional Nic-KLH conjugate vaccine.

2. Experimental section

2.1. Materials

Lactel® 50:50 PLGA was purchased from Durect Corporation (Cupertino, CA). Fetal bovine serum (FBS), granulocyte macrophage-colony stimulating factor (GM-CSF) recombinant mouse protein, alpha minimum essential medium, trypsin/EDTA, and Alexa Fluor® 647 hydrazide were purchased from Life Technologies Corporation (Grand Island, NY). The anti-mouse IgG from goat, anti-mouse IgG_1, IgG_{2a}, IgG_{2b}, IgG_3 HRP, and anti-goat IgG-HRP were procured from Alpha Diagnostic Intl (San Antonio, TX). TMB one component microwell substrate was procured from SouthernBiotech (Birmingham, AL). Lipids, including 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG(2000) carboxylic acid), cholesterol, MPLA and 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (ammonium salt) (NBD PE) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Poly(vinyl alcohol) (PVA, MW 89,000–98,000), dichloromethane (DCM), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich Inc. (Saint Louis, MO). Alexa Fluor® 647 Hydrazide, KLH, Imject™ Alum Adjuvant (Alum), 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), and sulfo-NHS were purchased from Thermo Fisher Scientific Inc. (Rockford, IL). JAWSII (ATCC® CRL-11904™) immature dendritic cells were purchased from ATCC (Manassas, VA). Rac-trans 3′-aminomethyl nicotine was purchased from Toronto Research Chemicals Inc. (Toronto, Canada). All other chemicals were of analytical grade.

2.2. Synthesis of KLH-containing PLGA nanoparticles

PLGA nanoparticles were prepared using a reported double emulsion solvent evaporation method with modifications [19–21]. Briefly, PLGA (30 mg) was dissolved in DCM (1 mL), followed by mixing with 100 μL of KLH (20 mg/mL) for 2 min using a vortex mixer. The resultant mixture was emulsified in a Branson B1510DTH Ultrasonic Cleaner (Branson, Danbury, CT) for 10 min. The primary emulsion was added drop-wise into 100 mL PVA (0.5% (w/v)), and continuously stirred for 10 min at 500 rpm. The above suspension was emulsified by sonication using a sonic dismembrator (Model 500; Fisher Scientific, Pittsburg, PA) at 50% amplitude for 120 s. The secondary emulsion was stirred overnight to allow DCM to evaporate. Large particles were removed after the mixture sat undisturbed at room temperature for 30 min. Nanoparticles in suspension were collected by centrifugation at 10,000 *g*, 4 °C for 60 min using an Eppendorf centrifuge (Eppendorf, Hauppauge, NY). The pellet was suspended in 10 mL phosphate buffered saline (PBS) buffer (pH 7.4) and stored at 2 °C until future use.

2.3. Assembly of NanoNiccine

Lipid-PLGA nanoparticles were assembled using a method as described in previous reports [20,22]. The lipid film containing 0.25 mg MPLA, 2.83 mg DOTAP, 3.08 mg DSPE-PEG(2000) carboxylic acid, and 0.1 mg cholesterol was hydrated with 1 mL of 55 °C pre-warmed PBS buffer. The resulting liposome suspension was vigorously mixed using a vortex mixer for 2 min, followed by sonication for 5 min, using a Branson B1510DTH Ultrasonic Cleaner (Branson, Danbury, CT) and then cooled to room temperature. The prepared liposome was added into the above prepared KLH-containing PLGA nanoparticles and pre-homogenized for 15 min using a Branson B1510DTH Ultrasonic Cleaner, followed by sonication for 5 min in an ice bath using a sonic dismembrator at 15% amplitude (pulse on 20 s, pulse off 50 s). The acquired lipid-PLGA nanoparticles were dialyzed against 500 mL activation buffer (0.1 M MES, 0.5 M NaCl, pH 6.0) for 2 h. EDC (4.1 mg) and sulfo-NHS (11.3 mg) were added into the hybrid nanoparticle suspension and allowed to react for 20 min at room temperature. The activated nanoparticles were dialyzed against 1000 mL PBS buffer (100 mM sodium phosphate, 150 mM NaCl; pH 7.2) for 30 min. After dialysis, 4.1 mg rac-trans 3′-aminomethyl nicotine was incubated with the above nanoparticle suspension at room temperature for 4 h. The remaining impurities were removed by dialysis against PBS buffer (pH 7.4) for 12 h. The assembled NanoNiccine was stored at 4 °C until future use.

2.4. Synthesis of nicotine-KLH conjugate vaccine

KLH (4 mg) dissolved in 2 mL activation buffer (0.1 M MES, 0.5 M NaCl, pH 6.0) was incubated with 1 mg EDC and 2.8 mg sulfo-NHS for 20 min. The activated KLH was transferred to an Amicon Ultra 15 mL centrifugal filter unit (NMWL, 50 KDa), and purified by centrifugation at 5000 *g* for 20 min. The purified KLH was suspended in 2 mL PBS buffer (100 mM sodium phosphate, 150 mM NaCl; pH 7.2) and reacted with 1 mg rac-trans 3′-aminomethyl nicotine at room temperature for 4 h. The resultant mixture was then transferred to the centrifugal filter unit mentioned above and centrifuged at 5000 *g* for 20 min to remove free nicotine. The purified nicotine-KLH conjugate was suspended in 2 mL PBS buffer (pH 7.4) and stored at 4 °C until future use.

2.5. Synthesis of nicotine-bovine serum albumin (Nic-BSA) conjugate

Bovine serum albumin (BSA) (10 mg) dissolved in 5 mL activation buffer (0.1 M MES, 0.5 M NaCl, pH 6.0) was incubated with 2 mg EDC and 5.6 mg sulfo-NHS for 20 min. The activated BSA was transferred to an Amicon Ultra-15 Centrifugal Filter Unit (NMWL, 30 KDa), and purified by centrifugation at 5000 *g* for 20 min. The purified BSA was suspended in 5 mL PBS buffer (100 mM sodium phosphate, 150 mM NaCl; pH 7.2) and reacted with 2 mg rac-trans 3′-aminomethyl nicotine at room temperature for 4 h. The resultant mixture was then transferred to the centrifugal filter unit mentioned above and centrifuged at 5000 *g* for 20 min to remove free nicotine. The purified nicotine-KLH conjugate was suspended in 5 mL PBS buffer (pH 7.4) and stored at 4 °C until future use.