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Rational incorporation of molecular adjuvants into a hybrid nanoparticle-based nicotine vaccine for immunotherapy against nicotine addiction

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ABSTRACT

Current clinically-tested nicotine vaccines have yet shown enhanced smoking cessation efficacy due to their low immunogenicity. Achieving a sufficiently high immunogenicity is a necessity for establishing a clinically-viable nicotine vaccine. This study aims to facilitate the immunogenicity of a hybrid nanoparticle-based nicotine vaccine by rationally incorporating toll-like receptor (TLR)-based adjuvants, including monophosphoryl lipid A (MPLA), Resiquimod (R848), CpG oligodeoxynucleotide 1826 (CpG ODN 1826), and their combinations. The nanoparticle-delivered model adjuvant was found to be taken up more efficiently by dendritic cells than the free counterpart. Nanovaccine particles were transported to endosomal compartments upon cellular internalization. The incorporation of single or dual TLR adjuvants not only considerably increased total anti-nicotine IgG titers but also significantly affected IgG subtype distribution in mice. Particularly, the nanovaccines carrying MPLA+R848 or MPLA+ODN 1826 generated a much higher anti-nicotine antibody titer than those carrying none or one adjuvant. Meanwhile, the anti-nicotine antibody elicited by the nanovaccine adjuvanted with MPLA+R848 had a significantly higher affinity than that elicited by the nanovaccine carrying MPLA+ODN 1826. Moreover, the incorporation of all the selected TLR adjuvants (except MPLA) reduced the brain nicotine levels in mice after nicotine challenge. Particularly, the nanovaccine with MPLA+R848 exhibited the best ability to reduce the level of nicotine entering the brain. Collectively, rational incorporation of TLR adjuvants could enhance the immunological efficacy of the hybrid nanoparticle-based nicotine vaccine, making it a promising next-generation immunotherapeutic candidate for treating nicotine addiction.

1. Introduction

Tobacco smoking has constantly been one of the largest public health concerns worldwide for decades. It is the leading cause of preventable diseases and premature deaths, and results in huge socioeconomic burdens [1,2]. In recent decades, nicotine vaccines have been studied as a promising immunotherapeutic strategy to combating nicotine addiction [3,4]. In principle, nicotine vaccines can induce the production of nicotine-specific antibodies that can bind with nicotine in serum and thus keep nicotine from entering the brain [5]. To date, numerous conjugate nicotine vaccines (CNVs) have been reported to achieve high immunological efficacy in preclinical trials, and some of them have entered various stages of clinical trials [6–9]. However, none of these clinically tested CNVs have shown improved overall smoking cessation rate compared to the placebo, mainly due to the insufficient titers of antibodies and their low binding capacity [5,10].

Immunologically, the immune system prefers to recognize particulate antigens and is relatively invisible to soluble protein antigens [11,12]. Therefore, the insufficient immunogenicity of conventional CNVs can be partially attributed to their intrinsic shortfalls, such as poor recognition and internalization by immune cells and low bioavailability. In addition, even with the help of alum

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to form particulate particles, CNVs cannot be easily tuned to have optimal physicochemical properties (such as size, shape, and charge) for cellular uptake [13,14]. Moreover, molecular adjuvants cannot be easily incorporated into CNVs, and they are typically co-administered with CNVs via physical mixing. In this way, molecular adjuvants are not specifically available to immune cells and their release cannot be controlled in immune cells, thus leading to low adjuvant efficacy and systemic toxicity [13,15,16].

In our previous study, by taking advantage of the superiorities of nanoparticles (NPs), such as particulate nature, tunable physicochemical properties, and controlled payload release, we developed a lipid-polymeric hybrid nanoparticle (NP)-based nicotine vaccine (NanoNicVac) as a next-generation immunotherapeutic strategy against nicotine addiction [17,18]. We demonstrated that NanoNicVac had a significantly higher immunogenicity than the conjugate vaccine, and its immunological efficacy could be enhanced by modulating NP size [17], hapten localization [19], hapten density [20], and stimulating proteins (unpublished data).

From the immunological point of view, adjuvants are an important component of a vaccine formulation, and they are necessary for the induction of a strong immune response, especially for poorly-immunogenic antigens [15,21]. Currently, alum is the most-widely used adjuvant for vaccine development. However, alum has shown to be a relatively weak adjuvant and sometimes may cause lesions at injection sites [22,23]. Especially for NP-based vaccines, alum may absorb vaccine NPs to form very large particles, resulting in sizes that are not optimal for cellular uptake [14]. Also, due to the high viscosity, alum may disrupt the structure of vaccine NPs. In addition, alum can limit the release of vaccine NPs from injection sites and impair their availability to antigen presenting cells (APCs) [16,24]. Our previous studies suggested that the use of alum would not significantly improve the immunogenicity of NanoNicVac [20]. As alternatives, molecular adjuvants, such as toll-like receptor (TLR) agonists, have been studied as a class of promising potent adjuvants [25–27]. TLR agonists are capable of enhancing the secretion of cytokines, promoting the activation of antigen presenting cells, and enhancing the production of antibodies [28–30].

Based on the hypothesis that incorporation of appropriate molecular adjuvants may enhance the immunological efficacy of NanoNicVac, this study aims to further rationalize the design of NanoNicVac by developing a NanoNicVac particle capable of co-delivering nicotine antigens and TLR agonists. As shown in Fig. 1A, the nicotine-protein conjugates were conjugated to the surface of lipid-polymeric hybrid NPs for presentation. The lipid-shell and PLGA-core served as hosts for cell-surface-TLR and endosomal-TLR agonists, respectively. Monophosphoryl lipid A (MPLA) [31,32], Resiquimod (R848) [13,33], and CpG oligodeoxynucleotide 1826 (CpG ODN 1826) [34,35], all of which have been reported to significantly enhance immune responses, were selected as adjuvant candidates. In this study, NanoNicVac particles carrying different TLR adjuvants or combinations were fabricated, and their physicochemical properties were characterized. The cellular uptake of NanoNicVac particles was studied in dendritic cells. The immunogenicity and ability to reduce brain nicotine concentration of NanoNicVac were investigated in mice.

2. Materials and methods

2.1. Materials

1,2-Dioleoyl-3-trimethylammonium-propane (DOTAP), cholesterol (CHOL), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (ammonium salt) (NBD-PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG2000-maleimide), and MPLA were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Lactel® 50:50 poly(lactic-co-glycolic acid) (PLGA) was purchased from Durect Corporation (Cupertino, CA, USA). O-Succinyl-3′-hydroxymethyl-(±)-nicotine (Nic) was purchased from Toronto Research Chemicals (North York, ON, Canada). Keyhole limpet hemocyanin (KLH), Alexa Fluor® 647 NHS ester (AF647), coumarin-6 (CM-6), 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), and N-hydroxysulfosuccinimide (Sulfo-NHS) were purchased from Thermo Fisher Scientific (Rockford, IL, USA). CpG ODN 1826 and R848 were purchased from InvivoGen (San Diego, CA, USA). All other chemicals were of analytical grade.

2.2. Fabrication of adjuvant-loaded PLGA NPs

Adjuvant-loaded PLGA NPs were fabricated using a water/oil/water double-emulsion-solvent-evaporation method [17]. In brief, 40 mg of PLGA was dissolved in 2 mL of dichloromethane (DCM) to form an organic phase. For CpG ODN 1826- or R848-encapsulated PLGA NP preparation, 1.20 mg of CpG ODN 1826 in 200 μL of DI water or 1.50 mg of R848 in 200 μL of DI water-DMSO (9:1) was added into the organic phase. For CpG ODN 1826 and R848 co-encapsulated PLGA NP preparation, 1.20 mg of CpG ODN 1826 in 100 μL of DI water and 1.50 mg of R848 in 100 μL of DI water-DMSO (9:1) were added into the organic phase. The water-in-oil solution was mixed and emulsified by sonication for 10 min using a Branson M2800H Ultrasonic Bath Sonicator (Danbury, CT, USA). The resultant primary emulsion was added dropwise to 12 mL of 0.5% w/v poly(vinyl alcohol) solution under continuous stirring. The suspension was emulsified again by sonication using a sonic dismembrator (Model 500; Fisher Scientific, Pittsburg, PA, USA) at an amplitude of 70% for 40 s. The resultant secondary emulsion was stirred overnight to allow complete DCM evaporation. Blank PLGA NPs were prepared using a similar method, except that 200 μL of DI water was used as the first aqueous phase. Blank and adjuvant-loaded PLGA NPs were collected by centrifugation at 10,000 *g*, 4 °C for 30 min (Beckman Coulter Avanti J-251, Brea, CA, USA), washed three times, and stored at 4 °C for later use. To quantify the loading efficiency of R848 and ODN 1826, 20 mg of NPs were disrupted by incubating with 0.2 N NaOH for 14 h. After particles were completely dissolved, the solution was neutralized using 1 N HCl. The concentration of ODN 1826 was measured using a Quant-iT™ OliGreen™ ssDNA Assay kit (Thermo Fisher Scientific, Rockford, IL). The concentration of R848 was quantified by reverse-phase HPLC using a Luna C18 (2) reverse phase column. The loading efficiencies of adjuvants are shown in Table 1.

2.3. Preparation of lipid-PLGA hybrid NPs

Blank and MPLA-carrying liposomes were prepared using a lipid-film-hydration-sonication method as reported previously [17]. The lipid mixtures used for preparing blank and MPLA-carrying liposomes were composed of DOTAP, DSPE-PEG2000-maleimide, CHOL, and MPLA at molar ratios of 90:5:5:0 and 80:5:5:10, respectively. Lipid-PLGA hybrid NPs were fabricated using a sonication method as reported previously [17]. Particularly, 2.5 mg of liposomes were mixed with 25 mg of PLGA NPs for hybrid NP fabrication. Lipid-PLGA hybrid NPs were collected by centrifugation at 10,000 *g*, 4 °C for 30 min, washed three times, and stored at 4 °C for later use.

2.4. Assembly of NanoNicVac particles

Nic-KLH conjugates were synthesized using an EDC/NHS mediated reaction as reported previously [20]. NanoNicVac particles were