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Rationalization of a nanoparticle-based nicotine nanovaccine as an effective next-generation nicotine vaccine: A focus on hapten localization

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ABSTRACT

A lipid-polymeric hybrid nanoparticle-based next-generation nicotine nanovaccine was rationalized in this study to combat nicotine addiction. A series of nanovaccines, which had nicotine-haptens localized on carrier protein (LPKN), nanoparticle surface (LPNK), or both (LPNKN), were designed to study the impact of hapten localization on their immunological efficacy. All three nanovaccines were efficiently taken up and processed by dendritic cells. LPNKN induced a significantly higher immunogenicity against nicotine and a significantly lower anti-carrier protein antibody level compared to LPKN and LPNK. Meanwhile, it was found that the anti-nicotine antibodies elicited by LPKN and LPNKN bind nicotine stronger than those elicited by LPKN, and LPNK and LPNKN resulted in a more balanced Th1-Th2 immunity than LPKN. Moreover, LPNKN exhibited the best ability to block nicotine from entering the brain of mice. Collectively, the results demonstrated that the immunological efficacy of the hybrid nanoparticle-based nicotine vaccine could be enhanced by modulating hapten localization, providing a promising strategy to combatting nicotine addiction.

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1. Introduction

Tobacco smoking is one of the most significant public health threats the world has ever faced; approximately, 6 millions of premature deaths are attributed to tobacco use each year in the world [1–3]. Despite the strong desires to quit smoking, the majority of unassisted smokers usually relapse within the first month, and only 3–5% of them remain abstinent after 6 months [4]. Even with the help of pharmacological interventions, including nicotine replacement therapy, varenicline, and bupropion, the long-term smoking cessation rate at one year is disappointingly low (10–25%) [5–8].

Nicotine vaccine has shown to be an attractive approach for smoking cessation [9,10]. Promisingly, some conjugate nicotine vaccines were successful in inducing strong immunogenicity as well as achieving high pharmacokinetic efficacy in preclinical and early-stage clinical trials [11–14]. However, none of them have shown overall enhanced smoking cessation rate over placebo so far, mainly due to the highly-varying and insufficient antibody titers [15–17]. Although great efforts have been made to improve their immunogenicity by modulating multiple factors [13,18–23], conjugate nicotine vaccines bear some intrinsic shortfalls, such as fast degradation, low nicotine loading capacity, low bioavailability, and poor recognition and uptake by immune cells, largely limiting their immunological efficacy.

To circumvent these disadvantages of conjugate nicotine vaccines, in our previous work, we designed the next-generation nicotine nanovaccines using nanoparticles (NPs) as delivery vehicles for antigen presentation [24–26]. Particularly, lipid-polymeric hybrid nanoparticle (NP)-based nicotine nanovaccines were demonstrated to induce significantly higher immunogenicity over the conjugate vaccines and result in better pharmacokinetic efficacy in mice [26,27].

Nicotine hapten is such a small molecule that can only elicit an immune response when attached to a carrier, such as proteins or

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nanoparticles [9]. In addition, a stimulating/carrier protein is always a necessity in NP-based nicotine vaccine, as it will stimulate T-helper cell formation that is required for B cell maturation [9,28]. Meanwhile, conjugating protein antigen to the surface of NPs could promote its delivery and presentation [29,30]. In our previous nanovaccine design, hapten was conjugated to the surface of protein antigens [26]. As the localization of haptens on vaccine NPs may potentially affect the recognition of antigens by immune cells, in this current work, we rationalized the design of a hybrid NP-based nicotine vaccine by studying the impact of hapten localization on its immunogenicity and pharmacokinetic efficacy. As shown in Scheme 1, three nanovaccines, which have haptens localized only on the carrier protein (**LPKN**), only on NP surface (**LPNK**), or on both (**LPNKN**), were synthesized. The immunogenicity and pharmacokinetic efficacy of nanovaccines were tested in mice.

2. Materials and methods

2.1. Materials

Lactel® (50:50 poly(lactic-*co*-glycolic acid) (PLGA)) was purchased from Durect Corporation (Cupertino, CA, USA). Keyhole limpet hemocyanin (KLH) was purchased from Stellar Biotechnologies (Port Hueneme, CA, USA). Alexa Fluor® 647 NHS ester (AF647), Alexa Fluor® 350 NHS ester (AF350), 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), and N-hydroxysulfosuccinimide (Sulfo-NHS) were purchased from Thermo Fisher Scientific (Rockford, IL, USA). 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 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(-polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG2000-amine) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). O-Succinyl-3'-hydroxymethyl-(±)-nicotine (Nic) was purchased from Toronto Research Chemicals (North York, ON, Canada). All other chemicals were of analytical grade.

2.2. Fabrication of PLGA NPs by nanoprecipitation

PLGA NPs were fabricated by a nanoprecipitation method [31]. In brief, 20 mg of PLGA was dissolved in 2 mL of acetone. The PLGA-in-acetone organic solution was injected into 10 mL of 0.5% PVA aqueous phase by a vertically mounted syringe pump with magnetic stir agitation (1200 rpm). The resultant suspension was placed under vacuum for 6 h to eliminate the organic solvent. PLGA NPs were collected by centrifugation at 10,000 *g*, 4 °C for 30 min.

2.3. Fabrication of lipid-polymeric hybrid NPs

Lipid-polymeric hybrid NPs were fabricated with a previously reported hydration-sonication method [26,32]. In brief, 2.5 mg of lipid mixture consisting of different molar ratios of DOTAP, DSPE-PEG2000-maleimide, DSPE-PEG2000-amine, and CHOL, was evaporated to form a lipid film. The lipid film was hydrated with 0.01 M phosphate buffer saline (PBS) and sonicated for 2 min to form a liposome suspension. Lipid-polymeric hybrid NPs were assembled by coating liposomes to PLGA NPs (PLGA: lipids = 10:1 (w/w)) via sonication for 10 min. Lipid-polymeric hybrid NPs were collected by centrifugation at 10,000 *g*, 4 °C for 30 min. The PLGA cores were labeled by Nile Red, and the number of NPs per mg was estimated by flow cytometry using an Amnis ImageStream^X Mark 2 imaging flow cytometer.

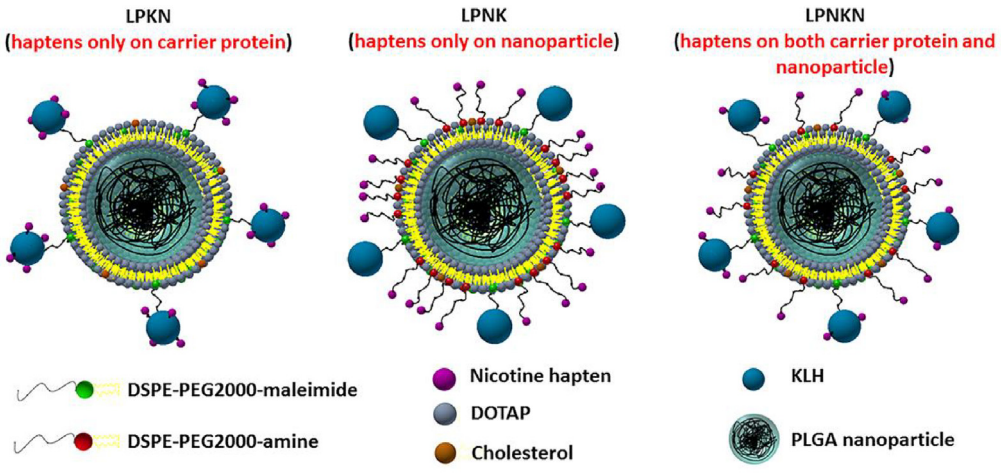
2.4. Synthesis of Nic-KLH conjugates

Nic-KLH conjugates were synthesized by an EDC/NHS-mediated reaction as reported previously [26]. Specifically, the Nic-KLH conjugates used for preparing LPKN or LPNKN nanovaccines were synthesized by reacting 1.2 mg or 2.4 mg of Nic hapten with 5 mg of KLH. Hapten densities of Nic-KLH conjugates were estimated by a 2,4,6-trinitrobenzene sulfonic acid-based method as reported previously [33]. Nic-BSA conjugate was synthesized using the same method.

2.5. Preparation of nanovaccine NPs

LPKN nanovaccine NPs were assembled with the method reported previously [26]. In brief, an appropriate amount of Traut's reagent was added into the Nic-KLH conjugates equivalent to 2 mg of KLH in 0.5 mL of PBS and reacted for 1 h to form thiolated Nic-KLH. The thiolated Nic-KLH equivalent to 1 mg of KLH was conjugated to 30 mg of lipid-polymeric hybrid NPs by reacting the thiolated Nic-KLH with maleimide groups in the lipid layer of NPs for 2 h. Unconjugated Nic-KLH was separated by centrifugation at 10,000 *g*, 4 °C for 30 min, and quantified by the bicinchoninic acid assay. Negative control was prepared following a similar procedure, except that KLH, instead of Nic-KLH, was conjugated to NP surface.

For LPNK and LPNKN synthesis, Nic-haptens were conjugated to



Scheme 1. Schematic illustration of the structure of hybrid NP-based nicotine nanovaccines with different hapten localizations.

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