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Optimization of a multivalent peptide vaccine for nicotine addiction

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ABSTRACT

We have been optimizing the design of a conjugate vaccine for nicotine addiction that employs a peptide-based hapten carrier. This peptide, which is produced by solid-phase protein synthesis, contains B cell and T cell epitope domains and eliminates the non-relevant, but highly immunogenic sequences in microbial carriers. In this report, the amino acid sequences in the T cell domain were optimized for improved vaccine activity and multivalent formulations containing structurally distinct haptens were tested for the induction of additive antibody responses. Trivalent vaccines produced antibody concentrations in mice that were 100 times greater than the amount of nicotine measured in smokers, and significantly reduced acute nicotine toxicity in rats. Two additional features were explored that distinguish the peptide from traditional recombinant carriers. The first is the minimal induction of an anti-carrier response, which can suppress nicotine vaccine activity. The second employs solid-phase synthesis to manufacture haptenated peptide. This approach obviates conventional conjugation chemistries and streamlines production of a more potent vaccine antigen.

1. Introduction

Drug addiction is a chronic relapsing brain disease that drives compulsive craving, loss of control, and continued use despite the negative consequences [1]. Substance use disorders account for approximately 11% of the total disease burden globally, and the World Mental Health Survey conducted by the WHO reported a prevalence rate of nearly 3% across 26 countries [2]. Unfortunately, the number of effective medications for treating addiction is very limited, due in large part to the complexity in developing safe pharmaceuticals that inhibit or modulate specific neuronal pathways. An alternative therapeutic strategy addresses the role that pharmacokinetics of addictive drugs play in stimulating addiction. This approach uses antibodies (Abs) to prevent or slow drug entry into the brain so that the positive reinforcement from drug ingestion can be diminished. A variety of preclinical anti-drug vaccines and monoclonal Abs have been effective in diminishing physiological and behavioral responses associated with addiction in animals [reviewed in [3–5]]. However, testing in humans has proven difficult because the conjugate vaccines used in these studies failed to induce target Ab concentrations in most subjects.

We are developing a nicotine vaccine that uses a short synthetic peptide carrier in lieu of a traditional recombinant protein [6]. The first 35 amino acids (AA) form an amphipathic alpha-helix that mediates coiled-coil assembly and is used for high density hapten conjugation and antigen presentation. This B cell epitope domain is followed by universal CD4 T cell epitopes (TCEs) that broadly bind major histocompatibility complex II (MHC II) alleles across disparate species and human populations [7,8]. Using this carrier, we previously established the importance of GLA-SE adjuvant in boosting antibody responses, and the roles played by enantiopure haptens and bivalent formulations in regulating Ab concentration and affinity [6,9]. Here we set out to maximize vaccine performance even further by optimizing TCE domain composition and by formulating a vaccine with three structurally distinct haptens. This optimized vaccine induced an Ab concentration in mice that was 100x greater than amounts of nicotine measured in smokers [10] and reduced acute nicotine toxicity in rats. We also highlighted two features of the peptide that distinguish it from a recombinant carrier; induction of a poor anti-carrier Ab response that lessens the potential for carrier suppression [11], and solid-phase synthesis of haptenated peptide, which simplifies production and improves antigen activity.

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2. Methods

2.1. Materials

All chemicals (unless otherwise specified) were purchased from VWR (Radnor, PA). Lyophilized recombinant CRM_{197} was purchased from Reagent Proteins (San Diego, CA). All peptides were synthesized by Bio-Synthesis, Inc. (Lewiston, TX). Following resin cleavage, peptides were purified by reverse phase HPLC and size exclusion chromatography. The four nicotine haptens used for this study include: hapten 1′; (2S)-N,N′-(disulfanediyldiethane-2,1-diyl)bis[4-(2-pyridin-3-ylpyrrolidin-1-yl)butanamide] [12], hapten 3′; *trans*-3-aminomethylnicotine [13], hapten 4; 4-aminopropylnicotine [14], and hapten 6; nicotine-6-hexanoic acid [6]. Haptens were synthesized by Life Chemicals (Vancouver, BC) and Albany Molecular Research Inc (Albany, NY) using reported methodologies with the following changes. Haptens 1′ and 3′ were synthesized as racemic mixtures. Briefly, hapten 3′ was then succinylated and hapten 1′ treated with methyl bromoacetate and subsequently deprotected to the free carboxylic acid with lithium hydroxide. Enantiomer separation via supercritical fluid chromatography was performed by Averica Discovery (Marlborough, MA) and enantiomer chirality was assigned using vibrational circular dichroism (Biotools Inc., Jupiter, FL). The hapten 6-lysine building block was prepared by first protecting the α-carboxylic acid of Fmoc-Lys-OH (Sigma Aldrich, St. Louis, MO) as a *tert*-butyl ester. The ε-amine of this intermediate was coupled to the terminal carboxylic acid of hapten 6 using HATU/DIPEA and subsequently hydrolyzed to yield the final compound. This compound was incorporated into the synthetic peptide using standard amino acid coupling methodology. Peptide conjugations and hapten quantitation were performed as reported previously, where it was determined that 3–4 haptens per peptide monomer yield optimal Ab responses [10]. CRM_{197} was conjugated with Hapten 1′ at a loading of 15 haptens per protein based on the findings that loadings of 11–18 molecules per protein elicit optimal immune responses with this carrier [15]. To generate antigens for ELISA, BSA was conjugated with 1000 equivalents of each hapten.

2.2. Animals

Animal experimentation was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, the US Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Female outbred CD-1 mice and Sprague-Dawley rats (Charles River Laboratories) were housed under pathogen-free conditions in the Infectious Disease Research Institute vivarium, which operates under a currently approved Assurance #A4337-01 and USDA certificate #91-R-0061. For immunizations, antigen was combined with GLA-SE adjuvant (5 μg GLA, 2 v/v % SE) that was provided by Immune Design Corp (Seattle, WA). Animals were immunized in each hind quadriceps muscle on days 0, 21, 42 and serum was collected on days 35 and 56 for measuring nicotine-specific Ab responses. Mice and rats were injected with 50 and 250 μL per leg, respectively.

2.3. Nicotine-induced behavioral responses and pharmacokinetics

Behavior was recorded in an open field laser-activated Omnitech Environmental Chamber (Omnitech Electronics, Inc. Columbus OH). Data was recorded using Fusion Software. Distance travelled was calculated based on number of horizontal beam breaks per minute over the course of the assay. To test nicotine responses in naïve control and vaccinated rats, animals first received a sham saline i.p. injection and baseline movement was measured for 30 min. One week later, rats were injected i.p. with 3 mg/kg nicotine hydrogen tartrate and movement was again recorded for 30 min. Clinical scores were measured within the first 5 min upon return to the chamber by 3 blinded individuals using a 1 to 5 scale where 0 = no effect; 1 = weakness or mild imbalance; 2 = severe imbalance; 3 = hypotonia, inability to walk; 4 = tremors, mild seizures; 5 = clonic/tonic seizures. Cumulative horizontal distance traveled was tabulated for each rat during the first 10 min of the experiment. After two weeks, rats received a second i.p. injection (0.3 mg/kg) of nicotine hydrogen tartrate. Animals were anaesthetized with isoflurane and then sacrificed 5 min later. Blood was collected via cardiac puncture for serum preparation and the brain was removed, weighed and flash frozen in liquid nitrogen. Nicotine was measured following tissue sample extraction by LC-MS/MS (Alturas Inc, Moscow ID).

2.4. Antibody assays

Midpoint titers and relative avidity were determined by ELISA as previously reported [10]. Titers for multivalent formulations were determined using plates coated with individual hapten-BSA reagents and hapten-BSA mixtures at 0.5 μg/mL each. Percent antibody cross-reactivity between haptens was determined by dividing the midpoint titers to heterologous plated haptens by the midpoint titer to the homologous hapten. Anti-carrier Abs were determined using unconjugated CRM_{197} or P10 using Nunc Maxisorp plates and Covabtest NHS-activated plates (Sapphire North America, Ann Arbor MI). Functional Abs were assessed using a nicotine binding assay. Sera (100 μL) were pooled from each immunization group and 90 μL aliquots were spiked (2 μL) with serially diluted nicotine to achieve final nicotine concentrations of 0.01–10,000 μM. These samples were then subjected to equilibrium dialysis against an equal volume of 1X PBS (92 μL) for 4 h using an HTD96b equilibrium dialysis setup (HTDialysis, Gales Ferry, CT). Aliquots from the serum and buffer sides of the dialysis membranes were removed and analyzed by LC-MS/MS (Alturas, Moscow, ID). Unbound nicotine was quantified by comparing peak intensities to an internal standard of d4-nicotine and an external standard curve.

Experimental data were analyzed using GraphPad Prism. Statistical significance of the difference between groups was calculated by Student's 2-tailed *t*-test on log-transformed data and between three or more groups by 1-factor analysis of variance (ANOVA) followed by post-hoc analysis.

3. Results

3.1. Optimization of the T cell epitope domain

As a precursor to building a multivalent vaccine, we screened the activity of several universal TCEs. Table 1 lists 7 epitopes that have shown broad responses in rodents, monkeys and humans [7,16,17]. The first experiment tested the difference in activity between carriers containing single (P12 and P13) or fused (P9) TCEs. Peptides were conjugated with hapten 1′ (Fig. 1), formulated with GLA-SE adjuvant and then injected into outbred CD-1 mice. As indicated in Fig. 2A, P12 and P13 induced, respectively, mean Ab titers of 3.0×10^5 and 1.5×10^5 by day 56, whereas P9 stimulated a more than additive mean Ab titer of 9.5×10^5. The relative Ab avidities for nicotine were indistinguishable between these peptides. To test whether other TCE fusions could improve antibody responses relative to P9, we immunized mice with P10, P14, and P15, conjugated with hapten 3′ (Fig. 3). In spite of considerable

Table 1
Universal T cell epitopes.

Peptide	TCE origin	Sequence	Reference
P9	PADRE + Diptheria Tx	AKFVAAWTLKAAA + QSIALSSLMVAQAIP	[16,7]
P10	Hepatitis B + Measles	FFLLTRILTIPQSLD + LSEIKGVIVHRLEGV	[17,7]
P12	Diptheria Tx	QSIALSSLMVAQAIP	[7]
P13	PADRE	AKFVAAWTLKAAA	[16]
P14	Influenza + Tetanus toxoid	PKYVKQNTLKLAT + QYIKANSKFIGITE	[7]
P15	Tetanus	FNNFTVSFWLRVPKVSASHLEQY	[7]

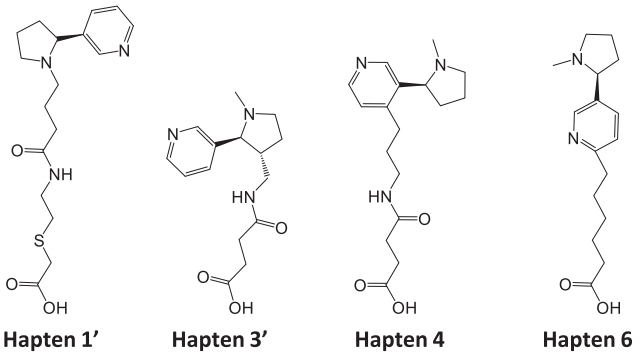

Fig. 1. Nicotine haptens used in this study (see Section 2).

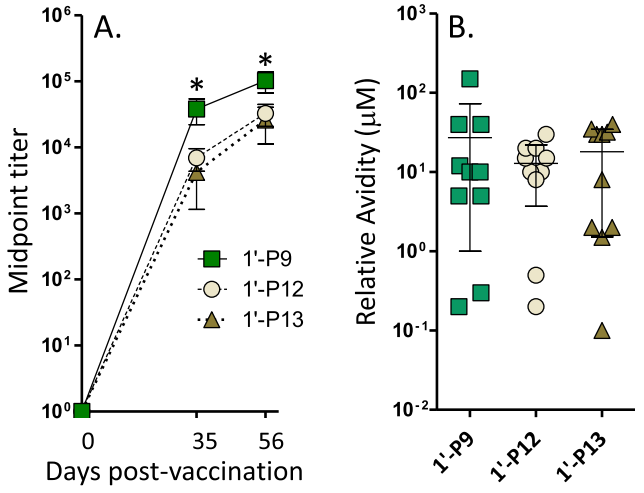

Fig. 2. TCE fusions synergistically improve Ab responses. Hapten 1′ was conjugated to P9, P12, or P13 (Table 1). Mice (n = 10/grp) were immunized with 5 μg peptide plus adjuvant. Serum was collected on d56 and assayed for anti-nicotine Ab titers (A) and relative nicotine avidities (B). Comparisons between groups were conducted by ANOVA. *p < 0.001.

overlap, 3′-P10 induced better Ab titers and avidities relative to the other peptides.

3.2. P10 peptides induce limited anti-carrier Ab responses

Recombinant protein carriers contain non-essential, highly immunogenic sequences that induce anti-carrier Abs, which can inhibit vaccine activity upon boosting. For instance, prior exposure of mice with CRM_{197}, the commercial carrier used in pneumonia, meningitis and Hib vaccines, significantly reduced the functional activity of a nicotine vaccine made with the same carrier [9]. Following selection of the P10 peptide, we compared its antigenic activity with CRM_{197} following conjugation with hapten 1′

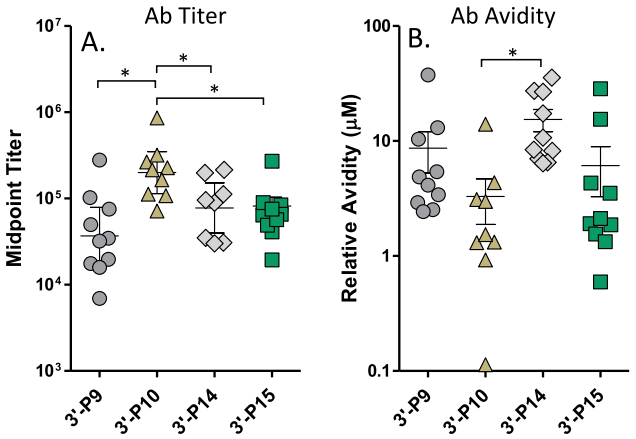

Fig. 3. P10 carrier selection. Peptides P9, P10, P14 and P15 (Table 1) were conjugated to hapten 3′ and mice were immunized with 5 μg of each peptide plus adjuvant. Ab titers (A) and relative Ab avidities (B) were assayed by ELISA using d56 sera. Comparisons between groups were conducted by ANOVA. *p < 0.05.

(Fig. 4). Both vaccines induced similar anti-nicotine titers and avidities. Interestingly, 1′-CRM_{197} stimulated equivalent anti-nicotine and anti-carrier Ab titers, whereas the anti-nicotine Abs induced by 1′-P10 were 3–4 orders of magnitude lower than anti-nicotine Abs (Fig. 4C). This result argues that P10 and its limited sequence complexity is much less likely to induce carrier suppression than a commercial microbial carrier.

3.3. Multivalent vaccine selection

The Pentel laboratory has shown that multivalent nicotine vaccines formulated with structurally-distinct haptens independently activate different B cell populations and stimulate additive Ab responses [12,18,19]. To use this approach, we first evaluated the relative immunogenicity of 4 enantiopure haptens that varied in linker length and nicotine attachment points (Fig. 1). As indicated in Fig. 5, all four haptens induced equivalent Ab titers, and except for hapten 4, similar Ab avidities and nicotine binding capacities. To gauge their suitability for multivalent formulations, we then measured the degree of antisera cross-reactivity to these four haptens; Ab binding between haptens 1′, 3′, and 6 were less than 10%, whereas cross-reactivity between haptens 3′ and 4 was approximately 50% (data not shown). This argues that haptens 1′, 3′ and 6 independently stimulate different B cell populations, whereas the B cell activation profiles of haptens 3′ and 4 overlap significantly.

To test how multivalency impacts circulating Ab concentrations, we immunized groups of mice with monovalent (3′), bivalent (1′+3′, 1′+4) or trivalent (1′+3′+4, 1′+3′+6) vaccines and determined nicotine binding capacities from day 56 sera (Fig. 6). The 1′+3′ and 1′+4 bivalent formulations induced a binding capacity that were ~1.75-2x better than the 3′ monovalent vaccine. Hapten 4 contribution to the 1′+3′+4 trivalent was marginal, which is due to its substantial Ab cross-reactivity with hapten 3′. Importantly, addition of hapten 6 to the 1′+3′+6 trivalent raised Ab concentration to 5.2 μg/mL sera, ~2.5 fold greater than the 3′ monovalent vaccine and ~100-fold greater than nicotine concentrations measured in smokers (20–60 ng/mL blood) [11]. These data confirm the strategy of using a multivalent vaccine for increasing circulating concentrations of anti-nicotine Abs.

3.4. Inhibition of nicotine-induced toxicity in rats

In parallel with testing multivalent vaccines in mice, we determined the immunogenicity of the 1′+3′+4 formulation in outbred

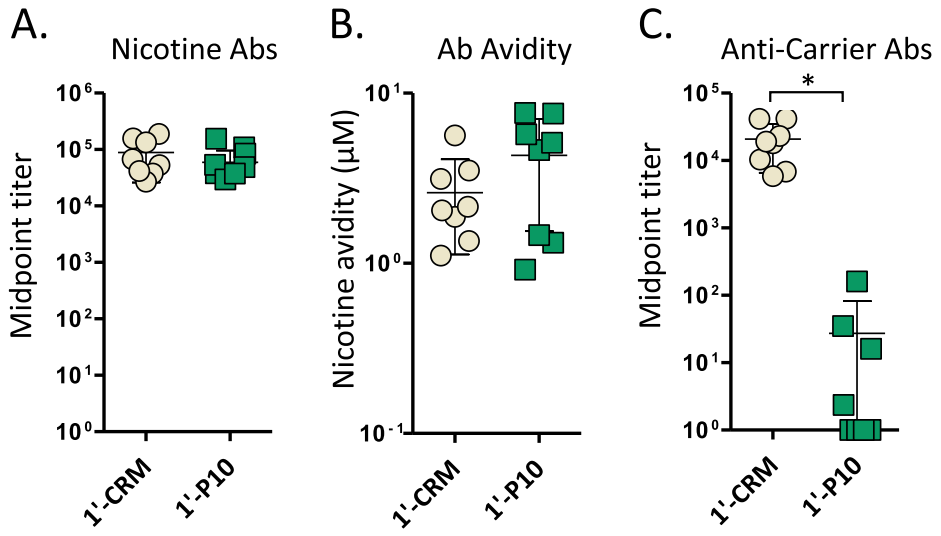

Fig. 4. P10 peptide induces dramatically lower anti-carrier Ab titers as compared to CRM_{197}. Mice (n = 8) were immunized with 10 μg of either 1′-P10 or 1′-CRM_{197} adjuvanted with GLA-SE. Anti-nicotine Ab titers (A), relative avidities (B), and anti-carrier Ab titers were assayed by ELISA using d56 sera. Comparison between groups were conducted by unpaired *t*-test. *p < 0.00001.

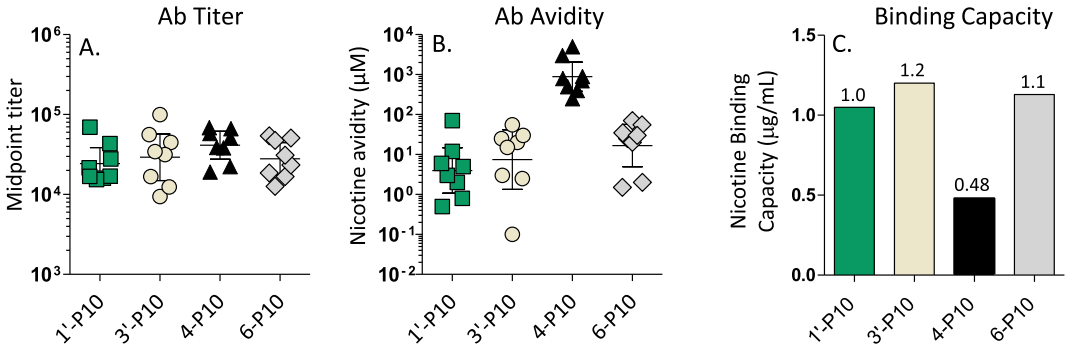

Fig. 5. Hapten activity comparison. The P10 peptide was conjugated to the indicated haptens, formulated with adjuvant, and then injected (2 μg) into mice. Day 56 sera was used to determine Ab titers (A), relative avidities (B), and nicotine binding capacities (C).

Sprague Dawley rats; the mean midpoint Ab titer in d56 sera (n = 12) was 2.8×10^5 and average nicotine binding capacity was 3.2 μg/mL. Animals were then subjected to an acute nicotine challenge. One week after measuring baseline locomotor activity, control and immunized rats received a large non-lethal dose of nicotine (3 mg/kg). Animals were then monitored for clinical signs of nicotine toxicity (Fig. 7A) and changes in locomotor activity (Fig. 7B). Nicotine exposure induced increasingly severe responses ranging from mild imbalance to seizures. A statistical difference (P < 0.006) was observed between groups; 10 of 12 control rats scored 3 or higher within minutes of nicotine injection while 8 of 12 immunized rats scored 2 or lower. The distance traveled by nicotine-challenged control rats was also significantly reduced compared to their baseline movement (p = 0.006) and to nicotine-injected immunized animals (p = 0.0001). In contrast, the immunized rats traveled the same average distance following PBS and nicotine injection. To corroborate these findings further, rats were rested an additional 2 week and then injected with nicotine (0.3 mg/kg i.p.). Brain and sera were collected 5 min later and then assayed for nicotine. As indicated in Fig. 8, vaccinated rat brain contained 60% less nicotine than control brains and 100-fold more nicotine was retained in sera. Collectively, these data show that the Abs induced in immunized rats can effectively sequester nicotine in blood and inhibit acute drug toxicity.

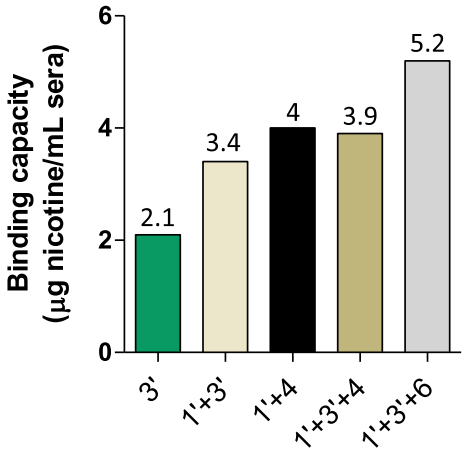

Fig. 6. Multivalent nicotine vaccines induce additive Ab responses. Monovalent, bivalent and trivalent vaccines were formulated with the indicated conjugates (5 μg each) plus adjuvant and then injected into mice (10/grp). Day 56 serum was used to determine average nicotine binding capacities per group.

3.5. Peptides synthesized with haptenated amino acids outperform conventional conjugates

Several issues related to conjugation reactions dampen vaccine activity including: perturbation of carrier structure, carriers with

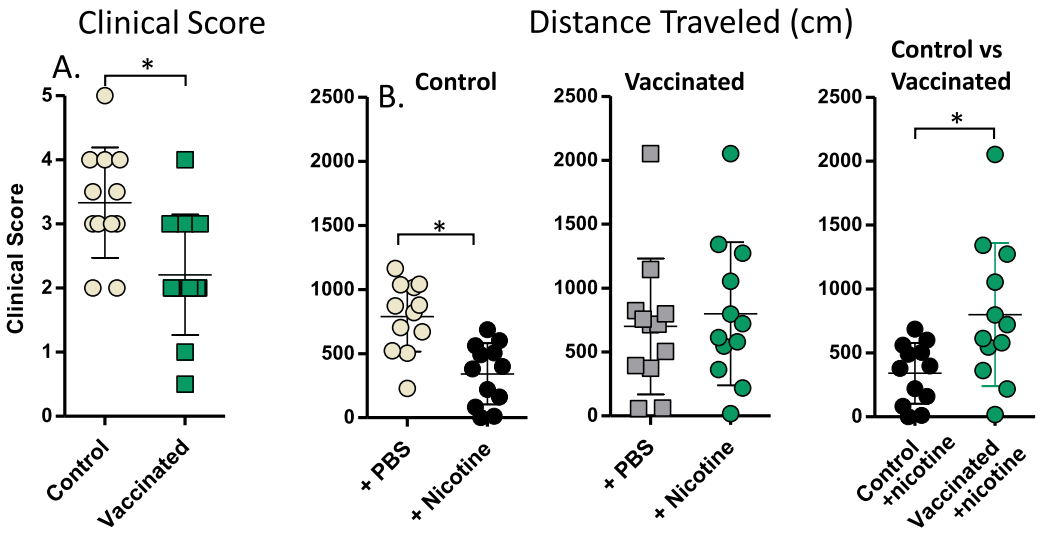

Fig. 7. Behavioral responses in rats following a nicotine challenge. Baseline mobility was measured in immunized and age-matched control rats after injection with PBS. One week later, animals were injected (i.p.) with 3.0 mg/kg nicotine, returned to chambers and measured in two ways (see Section 2). (A) Symptoms of nicotine toxicity using a 5-point scoring system (see Section 2). Comparisons between groups were conducted by unpaired *t*-test. *p = 0.006. (B) Horizontal distance traveled. Three comparisons are shown: control rats injected with PBS or nicotine, vaccinated rats injected with PBS or nicotine, and control versus immunized rats following nicotine injection. Comparisons between groups were conducted by unpaired *t*-test. For the control comparison between baseline and nicotine treatment, *p = 0.0001. For nicotine-treated control vs vaccinated groups, *p = 0.016.

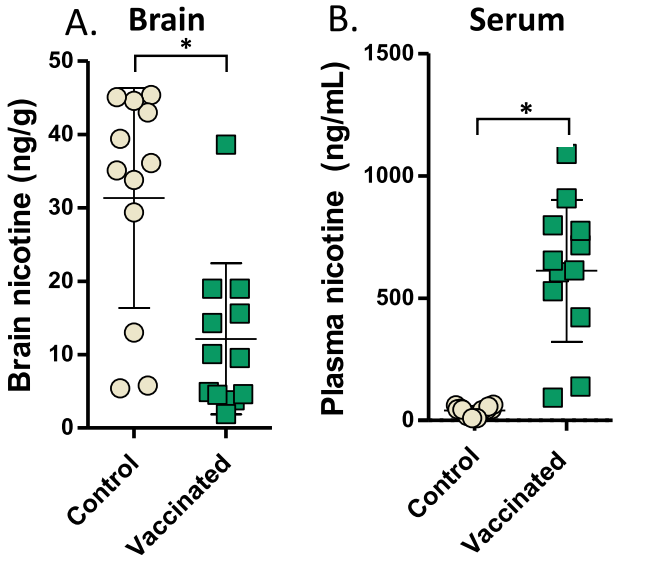

Fig. 8. Nicotine pharmacokinetics in control and vaccinated rats. Animals were injected (i.p.) with 0.3 mg/kg nicotine and tissues collected 5 min later. Nicotine levels were measured in brain and serum by LC/MS. Comparisons between groups were conducted by unpaired *t*-test. *p < 0.001.

variable hapten loadings, introduction of small molecular weight impurities, and aggregation [20,21]. However, since P10 is manufactured by solid phase protein synthesis, we can eliminate these problems by incorporating hapten-modified amino acids during the reaction [22]. Accordingly, a hapten 6-lysine building block (BB) was produced (Fig. 9), and peptides synthesized containing either 1 or 3 BBs in the outward-facing "f" position of the amphipathic heptad sequence (see Fig. 1, ref. [6]). Mice were then immunized and Ab responses to these peptides were compared to a conventional conjugated peptide (6-P10). As indicated, 6-P10 × 3BB induced the best titers and avidities, which more than doubled the resultant serum nicotine binding capacity relative to 6-P10, or the peptide that contained a single building block (6-P10 × 1BB). These results argue that synthesis of haptenated peptides produce a more homogenous and effective vaccine than conventionally-conjugated carriers.

4. Discussion

Addiction to nicotine and tobacco-related products is the single largest cause of cancer, heart disease and stroke, and half of the estimated 1.3 billion tobacco users in the world will die from their habit. Given the lack of effective therapies, nicotine vaccines have been explored as a treatment that might curb the pleasures of nicotine and motivate users to quit. However, despite clinical efforts, the Ab concentrations induced in smokers have been too low to gauge the effectiveness of this approach. To overcome this limitation, we optimized several design features of a peptide-based vaccine that resulted in strong functional Ab responses in mice and rats.

The vaccine carrier used for these studies has 2 functional domains. The lysine-rich α-helical domain used for hapten attachment contains 5 heptad repeats that mediate self-assembly of trimeric coiled coils [6]. Analytical ultracentrifugation and dynamic light scattering has confirmed that, prior to conjugation, these peptides self-assemble into higher order trimers with an average size of 20–30 nM (unpublished). The second domain contains 2 universal MHC class II binding peptides required for T cell-mediated antibody responses. Despite their extreme polymorphism, HLA alleles can be categorized into various supertype structures, each of which bind a repertoire of related complimentary peptides [23]. Several promiscuous or universal T-cell epitopes have been identified based on binding affinity across MHC II alleles [7,16,17,24]. To optimize the TCE domain, we tested the importance of epitope number (Fig. 2) and whether specific TCE fusions might be more active than others. Using outbred mice to model MHC II diversity, the results showed that peptides containing two epitopes induced more than additive Ab titers relative to peptides with one epitope. Given the mechanics of peptide processing, this could be due to the presentation of new junctional peptides generated between the two TCEs that contribute to class II supertype binding. These experiments also showed that the fused hepatitis B and measles virus

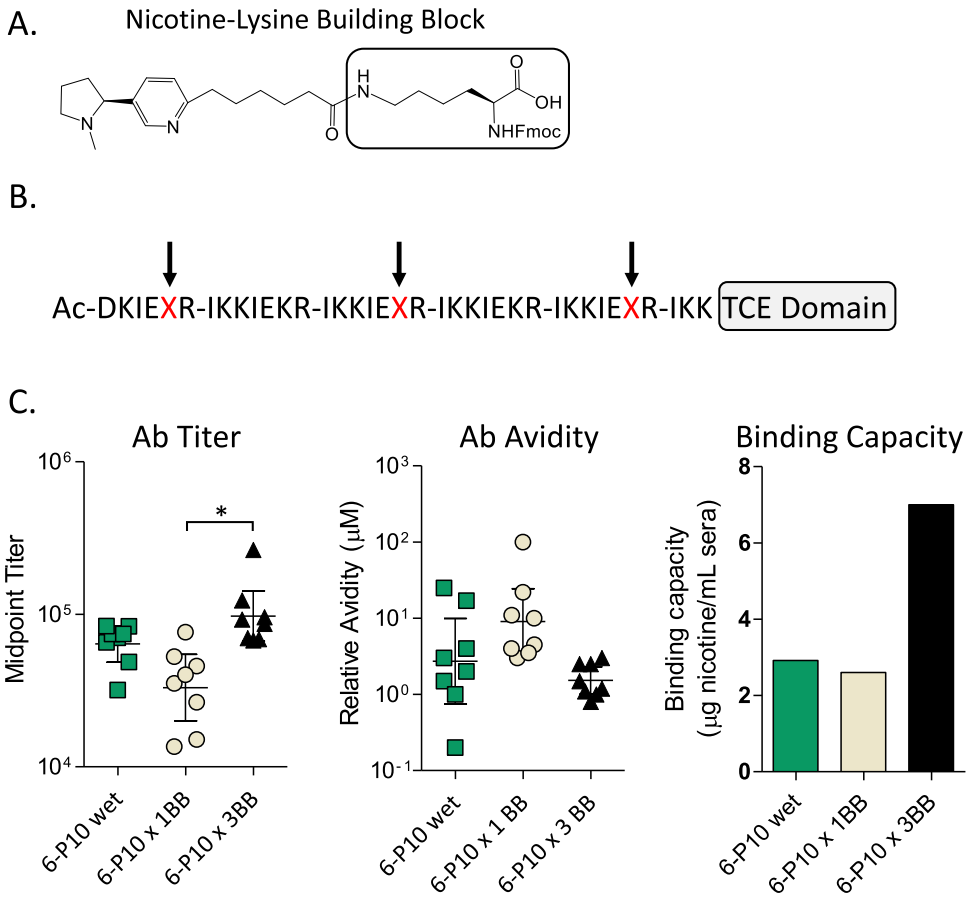

Fig. 9. Peptides synthesized with hapten building blocks induce superior Ab responses relative to conjugated peptides. (A) Structure of the hapten 6-lysine building block with an Fmoc protected side chain (outlined). (B) Peptide sequence showing building block insertion points (arrows). P10 peptides were synthesized with one BB positioned at the amino terminal location, BBs at all three sites or conjugated by conventional methods with an average 3.25 haptens per peptide. (C) CD-1 mice (n = 8) were immunized on days 0, 21 and 42 with 5 μg adjuvanted peptide and day 56 serum was assayed for Ab titer, avidity and average nicotine binding capacity. Comparisons between groups were conducted by unpaired *t*-test. *p < 0.008.

epitopes in P10 outperformed three other combinations in mice with respect to Ab titer and avidity.

An attractive feature of this peptide is the absence of irrelevant but highly immunogenic sequences common to traditional carrier proteins that induce anti-carrier Abs and subsequently suppress vaccine activity [25,26]. For example, McCluskie et al. have shown that prior exposure to CRM_{197} or Qb-VLP carriers inhibited the activity of two nicotine vaccines prepared with the same carrier [9]. To interrogate the anti-carrier response of P10, we compared its immunogenicity to CRM_{197} when conjugated with an optimal hapten load and formulated in the same adjuvant. Despite inducing comparable anti-nicotine Ab titers and relative avidities, 1′-P10 stimulated anti-carrier titers orders of magnitude lower than 1′-CRM_{197}. Thus, the potential for carrier suppression is significantly reduced with peptides carriers, at least during a prime-boost-boost immunization regimen.

Multivalent nicotine vaccines that contain structurally distinct haptens represent an important strategy for increasing functional antibody responses [12,18,19]. This was previously confirmed in our lab using enantiopure 1′ and 3′ haptens conjugated to P9 [10]. To expand this approach, we compared the activities of 4 individual haptens conjugated to P10 and observed that hapten 1′, 3′ and 6 showed similar behavior while hapten 4 was an outlier with respect to Ab avidity and binding capacity. When tested in combinations, the 1′+3′ and 1′+4 bivalent formulations showed similar additive Ab responses over a 3′ monovalent vaccine. However, the 1′+3′+4 trivalent failed to increase binding capacity beyond the bivalent formulations presumably due to the Ab cross-reactivity between haptens 3′ and 4. Conversely, the 1′+3′+6 trivalent, comprising haptens with <10% cross-reactivity, showed nearly triple additivity over the monovalent vaccine.

Preclinical studies have established that vaccines and monoclonal Abs targeting cocaine, methamphetamine, nicotine and opioids can successfully attenuate drug-induced behaviors in animals [3,4]. Here, we confirmed that Ab responses induced in immunized rats effectively reduced acute nicotine toxicity and prevented nicotine entry into brain. Within the first minute, control animals exhibited symptoms ranging from severe imbalance to seizures, whereas these acute responses were reduced significantly in vaccinated animals ($p = 0.006$). Although there was experimental overlap, control animals also showed significantly less mobility after nicotine exposure than the immunized rats ($p = 0.016$) and this impairment was even more apparent when compared to their baseline behavior ($p = 0.0001$). However, no difference in mobility was observed in vaccinated animals after PBS or nicotine injection. Following a subsequent nicotine injection, it was further shown that the Abs induced in immunized rats effectively sequestered nicotine in blood and prevented drug entry into brain ($p < 0.001$). Thus, confirming the vaccine's mechanism of action.

Careful examination of the hapten-carrier conjugation reaction has identified numerous variables that negatively impact vaccine activity, including hapten load, accumulation of small molecular weight adducts, and aggregation [15,21,22]. The conjugation reaction can also modify the tertiary structure of protein carriers, as in

the case of CRM_{197} where partially unfolded monomers induced diminished responses [22]. Consequently, clinical GMP manufacturing of a conventional nicotine vaccine will require thorough analytics and downstream processing. To avoid these pitfalls, we tested a new approach where nicotine hapten-lysine building blocks were used to synthesize carriers containing either 1 or 3 haptens at specified sites. The peptide with 3 building blocks induced better Ab titer, avidity, and nicotine binding capacity, which reflects the important role that hapten valency plays in B cell activation [27–29]. This peptide also induced higher titers, more consistent avidities, and substantially greater binding capacity than a conventional conjugated peptide. The improved activity of BB peptides is presumably because solid-phase synthesis avoids the deleterious effects associated with traditional vaccines and it generates an identical product unlike chemical conjugation reactions, which are stochastic in nature.

5. Conclusions

Preclinical studies suggest that vaccines directed against abused drugs can be safe and effective in diminishing addiction-related behaviors. Yet, the challenge has been to understand how to induce an antibody concentration in humans that is large enough to neutralize the doses of ingested drug. Nicotine concentrations in the arterial blood of smokers typically range between 20 and 60 ng/mL [11]. This report has highlighted several features of a new vaccine platform that stimulated a nicotine binding capacity in rodents of 3000–6000 ng/mL, a 100-fold excess over the theoretical binding capacity needed for efficacy in humans. Going forward, it will be critical to determine the extent to which this antibody response can be maintained in primates and people.

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Declaration of interest

All authors are employees of TRIA Bioscience Corp.

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