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# Investigations into the efficacy of multi-component cocaine vaccines

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ABSTRACT

Although cocaine addiction remains a serious health and societal problem in the United States, no FDA-approved treatment has been developed. Vaccines offer an exciting strategy for the treatment of cocaine addiction; however, vaccine formulations need to be optimized to improve efficacy. Herein, we examine the effectiveness of a tricomponent cocaine vaccine, defined as having its hapten (GNE) and adjuvant (cytosine-guanine oligodeoxynucleotide 1826, CpG ODN 1826) covalently linked via the immunogenic protein ovalbumin (OVA). The tricomponent vaccine (GNE-OVA-CpG 1826) and a vaccine of analogous, individual components (GNE-OVA+CpG ODN 1826) were found to similarly induce highly specific anticocaine antibody production in mice and block cocaine's stimulant effects in hyperlocomotor testing.



Cocaine abuse and addiction remain significant health and societal problems in the United States, as evidenced by a 1.6-fold increase in overdose deaths from 2010 to 2015.[1] According to the Drug Abuse Warning Network (DAWN), 505,224 of the approximately 1.3 million, or 1 in 3, emergency department visits relating to drug misuse/abuse were attributed to cocaine during 2011.[2] The National Survey on Drug Use and Health (NSDUH) reported that domestic cocaine use has remained stable, with an estimated 1.5 million monthly users in 2014.[3] At present, cocaine addiction is treated with behavioral therapy, including counseling, contingency management and therapeutic communities; however, maintaining abstinence is extremely difficult for addicted individuals.[4,5] Pharmacological interventions, such as small molecule therapeutics and vaccines, would offer those suffering cocaine addiction additional tools for overcoming withdrawal and relapse; however, no FDA-approved treatments for cocaine addiction are available at this time.[6,7] As a result, there is great need for more efficacious and reliable therapeutic options.

Immunopharmacotherapy, or vaccination against drugs of abuse, offers an exciting direction for next-generation cocaine addiction treatment.[8–10] In this strategy, administration of a drug-protein immunoconjugate activates the immune system to generate highly specific antibodies that effectively minimize drug penetration of the blood-brain-barrier, and thus, concentrations at the site of action.[11–13] Preclinical evaluation of vaccines for cocaine addiction have alluded to the potential successes of this approach; however, the single anticocaine vaccine to reach clinical trials, termed TA-CD, failed in phase III due to inconsistent efficacy among trial participants.[14–17] As a result, subsequent studies have sought to improve vaccine efficacy through optimization of the hapten, immunogenic protein, and/or adjuvant formulation, in addition to, method of delivery.[18–25]

In the course of anticocaine vaccine development, the Janda laboratory has disclosed a cocaine hapten, termed GNE, which reliably induces cocaine-specific antibodies when conjugated to an immunogenic protein.[18,21,23,26–31] To access GNE, a six-carbon aliphatic linker was appended to cocaine via an amide, in place of the methyl ester (Fig. 1). This chemical modification not only installed the linker necessary to decorate immunogenic protein with hapten, but improved stability of the cocaine analog towards hydrolysis *in vivo*. Further, we have demonstrated that cytosine-guanine oligodeoxynucleotide (CpG ODN 1826), a TLR9 agonist, and alum function as the most advantageous adjuvant combination, eliciting robust Th1 and Th2 anticocaine immune responses and safely enhancing immunoglobulin G (IgG) antibody titers.[19,32–37] CpG ODNs, which have been extensively studied as vaccine adjuvants in the context of infectious diseases and cancer, enhance antigen (Ag)-specific immune responses by mediating the internalization of Ag into antigen presenting cells (APCs) via receptor mediated endocytosis and stimulating NFκB signaling cascade upon TLR9 binding.[38–41] Although we have successfully employed anticocaine vaccines, whereby the GNE immunoconjugate, CpG

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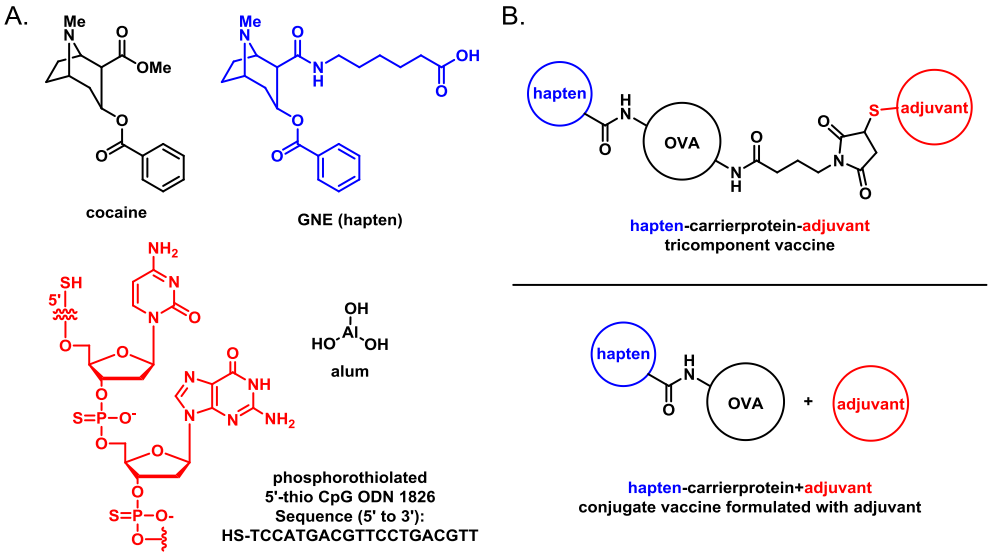


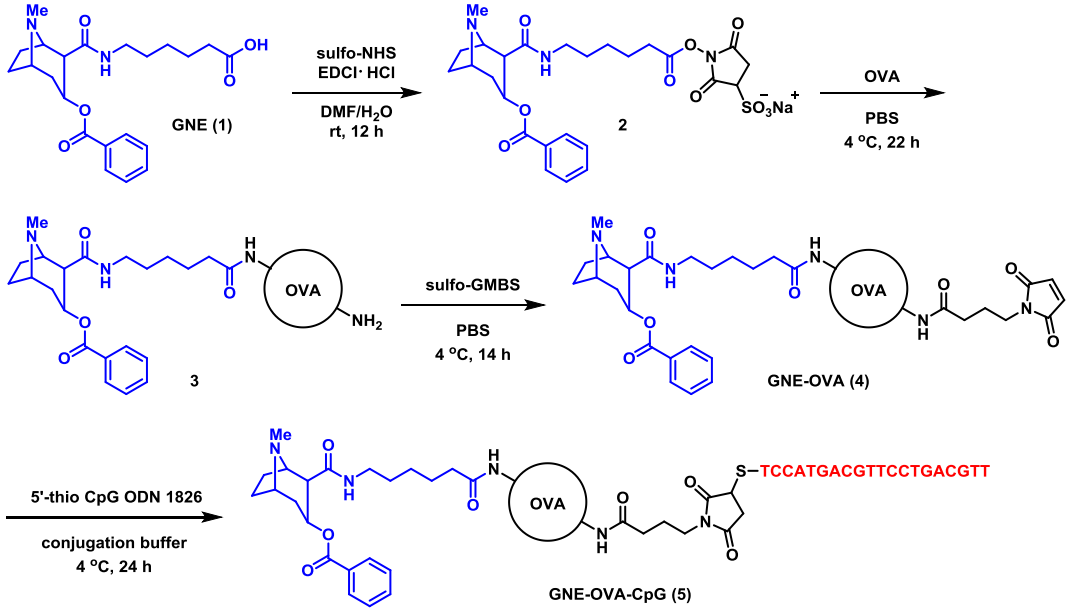

**Fig. 1.** Chemical structures of cocaine vaccine components presented in this study. (A) Individual vaccine components: cocaine, GNE (hapten), alum, and 5′-thio CpG ODN 1826. (B) Representative construction of tricomponent and conventional conjugate vaccines.

ODN 1826, and alum were mixed immediately prior to vaccination and delivered as individual components, numerous studies have reported significant boosts in vaccine efficacy upon co-internalization of the hapten and CpG ODN by the same APC (Fig. 1).[42–46]

To accomplish co-administration, the hapten and CpG ODN should be covalently linked. An augmented, Ag-specific immune response to covalent formulation is hypothesized to result from single APCs simultaneously presenting Ag and secreting cytokines. Specifically, endocytosis of CpG ODNs is mediated by DNA receptors that can also internalize Ag-linked CpG ODNs. Once internalized, the Ag is processed and presented to Th1 and Th2 cells by MHCII, while CpG ODN promotes the secretion of IL-12 upon TLR9 binding. It is this event, where single APCs concurrently present Ag and secrete IL-12, which promotes robust, Ag-specific, Th1-baised immune responses.[47,48] While significant evidence exists for this phenomenon, Ag-adjuvant conjugated vaccines for drugs of abuse have not been previously investigated. In this study, we disclose the first instance of a covalently conjugated, tricomponent (GNE-OVA-CpG ODN 1826) cocaine vaccine (Scheme 1).

Our synthesis of GNE-OVA and GNE-OVA-CpG immunoconjugates leveraged our third generation cocaine hapten, GNE, which was prepared from cocaine according to literature procedure.[25]

The carboxylic acid of GNE (**1**) was condensed with *N*-hydroxysulfosuccinimide (sulfo-NHS) using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) to give activated GNE **2**. Treatment of OVA with **2** in PBS enabled coupling of GNE to OVA lysine residues affording GNE conjugated OVA **3**. Unreacted lysine residues of **3** were cross-linked with *N*-γ-maleimidobutyryl-oxysulfosuccinimide ester (sulfo-GMBS) to give desired GNE-OVA (**4**). Finally, **4** was treated with reduced 5′-thio CpG ODN 1826 in Imject™ Maleimide Conjugation Buffer to yield GNE-OVA-CpG (**5**). Of important note, we employed OVA as the carrier protein to maximize hapten density and ease of MALDI-ToF characterization, despite its reduced immunogenicity compared to other immunogenic




**Scheme 1.** Synthesis of GNE-OVA-CpG tricomponent vaccine.