Published in final edited form as:

Bioorg Med Chem Lett. 2017 August 15; 27(16): 3666–3668. doi:10.1016/j.bmcl.2017.07.014.

Augmenting the efficacy of anti-cocaine catalytic antibodies through chimeric hapten design and combinatorial vaccination

Cody J Wenthur^a, Xiaoqing Cai^a, Beverly Ellis^a, and Kim D Janda^{a,*}

^aDepartments of Chemistry and Immunology, The Scripps Research Institute, La Jolla, CA, 92037, United States

Abstract

Given the need for further improvements in anti-cocaine vaccination strategies, a chimeric hapten (GNET) was developed that combines chemically-stable structural features from steady-state haptens with the hydrolytic functionality present in transition-state mimetic haptens. Additionally, as a further investigation into the generation of an improved bifunctional antibody pool, sequential vaccination with steady-state and transition-state mimetic haptens was undertaken. While GNET induced the formation of catalytically-active antibodies, it did not improve overall behavioral efficacy. In contrast, the resulting pool of antibodies from GNE/GNT co-administration demonstrated intermediate efficacy as compared to antibodies developed from either hapten alone. Overall, improved antibody catalytic efficiency appears necessary to achieve the synergistic benefits of combining cocaine hydrolysis with peripheral sequestration.

Graphical Abstract

Keywords

Vaccine; Cocaine; Catalytic Antibody; Chimeric Hapten; Addiction

Cocaine abuse and addiction are persistent public health problems in the US.¹ With millions of users reported annually, cocaine was the fifth-most consumed illicit drug type in 2014.² However, despite this prevalence, there have not yet been any medications approved by the FDA for the treatment of cocaine abuse or addiction.^{3,4} Among the strategies now being

*Corresponding Author. kdjanda@scripps.edu (K. D. Janda).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

explored for treatment of these conditions are antibody-based approaches, sometimes termed ‘cocaine vaccines’.^{5–7} These agents are designed to induce a drug-specific immune response in order to sequester cocaine in the periphery and block its psychoactive effects.^{8,9} Despite pre-clinical successes of this approach and promising efficacy signals in small-scale human studies, the overall efficacy of vaccination against cocaine in late-stage human trials has been limited thus far.^{10–13} Considering this challenge, substantial efforts have been made to improve the technology, including investigations into vaccine composition, adjuvant identity, delivery methods, and hapten design.^{14–21}

Within this search for improved hapten designs, our laboratory investigated the use of ‘catalytic haptens’ to chemically inactivate cocaine upon binding.²² In this study, while the use of GNT (**1**), a transition-state mimetic hapten, was able to generate antibodies that could both sequester and degrade cocaine, it also appeared that some of these antibodies underwent covalent modification by cocaine over time, thus reducing the vaccine’s ultimate effectiveness. The acylation appears to be due to activation of cocaine’s methyl ester, followed by nucleophilic attack from amino acids in the active site of the transition-state mimetic antibody. Therefore, in a subsequent investigation, we assessed the impact of hapten stability on the immunogenicity of the resulting vaccines. Intriguingly, replacement of this offending ester linkage with an amide was able to drastically increase the half-life of an analogous steady state hapten, GNE (**2**), which ultimately increased its immunogenicity.²³ Considering these findings, we postulated that the efficacy of a transition-state mimetic vaccine could be improved by promoting the selection of catalytic antibodies whose active site was directed away from recognizing and reacting with the methyl ester in cocaine.

Furthermore, recent findings have demonstrated the presence of synergistic gains in efficacy when cocaine hydrolyzing enzymes are directly administered together with antibodies.^{24,25} Given that the early clinical indications for applications of enzyme-mediated cocaine-hydrolysis are favorable, it seems highly prudent to direct efforts into further development and validation of additional therapeutic pairings that could combine the benefits of rapid cocaine catalysis and robust peripheral sequestration.^{26,27}

Therefore, in pursuit of improving the ‘catalytic’ vaccine strategy, we developed a new chimeric hapten that incorporated both the phosphate ester necessary to induce cocaine degradation and the amide linkage that improves hapten stability and eliminates covalent antibody modification by cocaine. This hapten, GNET (**3**), was generated using a convergent synthesis wherein cocaine was first boiled in 1.25 M HCl to quantitatively generate (–) ecgonine (**5**), which was then coupled with the amine linker species (**6**) using EDC/DMAP conditions to generate amide **7**. Linker **6** was prepared from Boc-6-aminohexanoic acid and intermediate **8** was prepared from treatment of phenylphosphoric dichloride with benzyl alcohol. Next, **7** was treated with LDA in the presence of **8** to produce intermediate **9**. Finally, global deprotection of **9** using hydrogenolysis provided GNET (**3**) in 18% overall yield (Scheme 1). The haptens **1–3** were then independently conjugated to Keyhole Limpet Hemocyanin (KLH) to generate the immunogens GNE-KLH, GNT-KLH, and GNET-KLH. Bovine serum Albumin (BSA) conjugates were also generated for use in biochemical analysis using the same method.

Each of these immunogens was then formulated with Alum and Sigma Adjuvant System (SAS) and administered subcutaneously (SC) to mice at 0, 3, and 6 weeks (Figure 1A). Analysis of antibody response at weeks 3 and 8 for each of these vaccines was then assessed using an Enzyme-Linked Immunosorbent Assay (ELISA), where titers were determined by measuring the binding of serum from vaccinated animals to their corresponding hapten-BSA conjugate. While all three vaccines generated an antibody response over this time period, the GNET-KLH group demonstrated the largest response by far (Figure 1B).

Next, we assessed the ability of purified antibodies from each of these vaccinated groups to catalyze the degradation of cocaine *in vitro* (Figure 1C). As anticipated, the antibodies generated by GNE showed no catalytic activity, while those generated by GNT were able to convert cocaine to methyl ecgonine and benzoic acid ($k_{\text{cat}} = 0.72 \pm 0.23 \text{ min}^{-1}$; $K_m = 235 \pm 104 \text{ }\mu\text{M}$). The antibodies from the GNET-treated animals demonstrated similar catalytic activity ($k_{\text{cat}} = 0.25 \pm 0.02 \text{ min}^{-1}$; $K_m = 38.3 \pm 7.83 \text{ }\mu\text{M}$) to that seen with GNT, although in both cases this activity was poor in comparison to naturally occurring enzymes.

When we measured the ability of vaccination to blunt hyperlocomotor activity due to cocaine administration, it was seen that GNE-KLH had the most robust response, with GNT-KLH having a lesser effect. Surprisingly, even though vaccination with GNET-KLH was generating active catalytic antibodies, it was not able to alter the animals' behavioral response to cocaine (Figure 1D).

Given the poor efficacy of this chimeric hapten strategy, we next attempted to determine whether the sequential administration of GNE and GNT could generate a superior outcome to that seen with GNET. We hypothesized that sequential administration could improve vaccination outcomes by incorporating a first round of selection for antibodies that had either limited methyl ester recognition or increased cocaine catalysis, and then incorporating a subsequent round of counter-selection for antibodies that retained recognition of the parent drug structure. For this study, the vaccination schedule was extended to four total injections in order to provide a balanced dose of GNE and GNT in the combination groups, but the total dose of hapten received over the course of the full study was not changed (Figure 2A). As a means to maximize the potential to identify differences between administration schedules for this combination in this round, we used a highly-immunogenic vaccine formulation, where the GNE and GNT haptens were conjugated to Tetanus Toxoid (TT) and administered with CpG Oligodeoxynucleotide 1826 (CpG) and alum.

Serum for ELISA analysis was taken after the second and fourth injections. Analysis of the serum collected from all the vaccination groups using ELISA revealed that the antibodies initially induced by GNE were not able to recognize GNT-BSA, and the antibodies initially induced by GNT were not able to recognize GNE-BSA. However, subsequent exposure to the opposing hapten did induce expansion of the antibody repertoire, although GNT-TT mainly generated an anti-GNT antibody pool, with little expansion of the existing population of anti-GNE antibodies. (Figure 2B).

Regardless of these changes, however, the order of GNE and GNT administration had no effect on the efficacy of the vaccines in blunting cocaine-induced hyperlocomotion. Each of

the combination schedules, including the concurrent administration of GNE and GNT, had an efficacy that fell between that of GNT-TT alone or GNE-TT alone (Figure 4C–D). This indicates that the overall efficacy of the combinations is primarily driven by the presence of independent reservoirs of sequestering and catalytic antibodies, and that there are no significant synergistic effects arising from altered affinity maturation during sequential hapten administration.

Using RIA, it was found that each of the combination schedules did result in the production of a set of stable cocaine-binding antibodies with affinities similar to that seen for GNE-TT vaccination alone (Figure 2D). Interestingly, the apparent antibody affinities for those schedules where GNT-TT was given earlier were lower than those where GNT-TT was given later. However, this shift is likely driven by lowered concentrations of cocaine in the RIA due to ongoing catalysis by established anti-GNT antibodies. Since transition-state mimetic antibodies result in the breakdown of cocaine over time, steady state binding studies can only provide a measure for the affinity and concentration of the non-catalytic antibody population (Table 1).

Overall, vaccination with GNE-TT was the most effective strategy attempted in this study, as compared to use of the structurally chimeric GNET hapten and the combinatorial dosing of GNT with GNE. Since both GNET and combination GNT dosing were able to induce measurable antibody responses, their ultimately limited behavioral efficacy is illuminating. These results indicate that improvement in the catalytic efficiency of transition-state mimetic haptens is essential if they ever hope to synergistically support steady-state hapten efficacy in a manner similar to that reported for hydrolytic enzymes directed against cocaine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Joel Schlosburg for helpful input on behavioral study design and analysis and Atsushi Kimishima for providing synthetic materials. Research reported in this publication was supported by the National Institute on Drug Abuse under grant R01DA008590-21. This is manuscript #XXXXXX from The Scripps Research Institute.

References

1. US Department of Health and Human Services Drug Abuse Warning Network (DAWN). 2011
2. US Department of Health and Human Services National Survey on Drug Use and Health. 2014
3. Kampman KM. *Addict. Sci. Clin. Pract.* 2008; 4:28–35. [PubMed: 18497715]
4. Czoty PW, Stoops WW, Rush CR. *Pharmacol. Rev.* 2016; 68:533–562. [PubMed: 27255266]
5. Martell BA, Mitchell E, Poling J, Gonsai K, Kosten TR. *Biol. Psychiatry.* 2005; 58:158–164. [PubMed: 16038686]
6. Zalewska-Kaszukska J. *Vaccine.* 2015; 33:6545–6551. [PubMed: 26432911]
7. Skolnick P. *Trends Pharmacol. Sci.* 2015; 36:628–635. [PubMed: 26435208]
8. Meijler MM, Matsushita M, Wirsching P, Janda KD. *Curr. Drug Discov. Technol.* 2004; 1:77–89. [PubMed: 16472221]
9. Kinsey B. *Ther. Adv. Vaccines.* 2014; 2:106–117. [PubMed: 24982760]

10. Kosten TR, Rosen M, Bond J, Settles M, Roberts JSC, Shields J, Jack L, Fox B. Vaccine. 2002; 20:1196–1204. [PubMed: 11803082]
11. Martell BA, Orson FM, Poling J, Mitchell E, Rossen RD, Gardner T, Kosten TR. Arch. Gen. Psychiatry. 2009; 66:1116–1123. [PubMed: 19805702]
12. Haney M, Gunderson EW, Jiang H, Collins ED, Foltin RW. Biol. Psychiatry. 2010; 67:59–65. [PubMed: 19846066]
13. Kosten TR, Domingo CB, Shorter D, Orson F, Green C, Somoza E, Sekerka R, Levin FR, Mariani JJ, Stitzer M, Tompkins DA, Rotrosen J, Thakkar V, Smoak B, Kampman K. Drug Alcohol Depend. 2014; 140:42–47. [PubMed: 24793366]
14. Maoz A, Hicks MJ, Vallabhjosula S, Synan M, Kothari PJ, Dyke JP, Ballon DJ, Kaminsky SM, De BP, Rosenberg JB, Martinez D, Koob GF, Janda KD, Crystal RG. Neuropsychopharmacology. 2013; 38:2170–2178. [PubMed: 23660705]
15. Cai X, Tsuchikama K, Janda KD. J. Am. Chem. Soc. 2013; 135:2971–2974. [PubMed: 23398531]
16. Ramakrishnan M, Kinsey BM, Singh RA, Kosten TR, Orson FM. Chem. Biol. Drug. Des. 2014; 84:354–363. [PubMed: 24803171]
17. Hicks MJ, Kaminsky SM, De BP, Rosenberg JB, Evans SM, Foltin RW, Andrenyak DM, Moody DE, Koob GF, Janda KD, Ricart Arbona RJ, Lepherd ML, Crystal RG. Hum. Gene Ther. Clin. Dev. 2014; 25:40–49. [PubMed: 24649839]
18. Lockner JW, Eubanks LM, Choi JL, Lively JM, Schlosburg JE, Collins KC, Globisch D, Rosenfeld-Gunn RJ, Wilson Ia, Janda KD. Mol. Pharm. 2015; 12:653–662. [PubMed: 25531528]
19. Rudra JS, Ding Y, Neelakantan H, Ding C, Appavu R, Stutz S, Snook JD, Chen H, Cunningham KA, Zhou J. ACS Chem. Neurosci. 2016; 7:546–552. [PubMed: 26926328]
20. Evans SM, Foltin RW, Hicks MJ, Rosenberg JB, De BP, Janda KD, Kaminsky SM, Crystal RG. Pharmacol. Biochem. Behav. 2016; 150–151:76–86.
21. Kimishima A, Wenthur CJ, Eubanks LM, Sato S, Janda KD. Mol. Pharm. 2016; 13:3884–3890. [PubMed: 27717287]
22. Cai X, Whitfield T, Hixon MS, Grant Y, Koob GF, Janda KD. J. Med. Chem. 2013; 56:3701–3709. [PubMed: 23627877]
23. Cai X, Whitfield T, Moreno AY, Grant Y, Hixon MS, Koob GF, Janda KD. Mol. Pharm. 2013; 10:4176–4184. [PubMed: 23927436]
24. Carroll ME, Zlebnik NE, Anker JJ, Kosten TR, Orson FM, Shen X, Kinsey B, Parks RJ, Gao Y, Brimijoin S. PLoS One. 2012; 7:e43536. [PubMed: 22912888]
25. Brimijoin S, Orson F, Kosten TR, Kinsey B, Shen XY, White SJ, Gao Y. Chem. Biol. Interact. 2013; 203:212–216. [PubMed: 22960160]
26. Cohen-Barak O, Wildeman J, van de Wetering J, Hettinga J, Schuilenga-Hut P, Gross A, Clark S, Bassan M, Gilgun-Sherki Y, Mendzelevski B, Spiegelstein O. J. Clin. Pharmacol. 2015; 55:573–583. [PubMed: 25524052]
27. Shram MJ, Cohen-Barak O, Chakraborty B, Bassan M, Schoedel KA, Hallak H, Eyal E, Weiss S, Gilgun-Serki Y, Sellers EM, Faulknor J, Spiegelstein O. J. Clin. Psychopharmacol. 2015; 35:396–405. [PubMed: 26082975]
28. For detailed information on methods used please see the supporting information.

Figure 1.

Ex vivo and in vivo measurements of antibody efficacy following vaccination with GNET.

A) Vaccination schedule for each hapten. B) Midpoint IgG titers as measured by ELISA (n = 6).

C) Michaelis-Menton plot for antibody-mediated breakdown of cocaine (n = 6). D)

Hyperlocomotor activity results from vaccinated animals treated with increasing doses of cocaine (n = 6).

Figure 2.

Ex vivo and in vivo measurements of antibody efficacy following combinatorial vaccination with GNE and GNT. A) Vaccination schedule for each condition, with dashed lines representing concurrent co-administration of GNE and GNT. B) Midpoint IgG titers against each hapten, as measured by ELISA ($n = 6$). C and D) Hyperlocomotor activity results from vaccinated animals treated with increasing doses of cocaine ($n = 6$).

Scheme 1.

Synthetic route to access GNET, a chimeric hapten combining features of GNE and GNT.

Conditions: a) 1.25 M HCl, 115 °C. b) BzOH, EDC, DMAP, DCM, 23 °C. c) TFA, DCM. d) EDC, DMAP, 4-methylmorpholine, DCM. e) BzOH, Pyridine, CHCl₃. f) LDA, THF. g) H₂, Pd/C, MeOH.

Table 1

Characteristics of resulting antibodies following vaccination as measured by RIA.

Group	GNE-TT	GNT-TT	E → T	T → E	E + T
Cocaine K _d (nM)	237 ± 97.2	-- [†]	285 ± 33.9	84.8 ± 28.9	72.3 ± 27.5
Concentration (ng/mL)	134 ± 116	-- [†]	154 ± 98.5	38.6 ± 18.9	66.4 ± 36.1

[†] Could not be determined due to complete catalytic breakdown of cocaine over the course of the assay