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A Bioconjugate Leveraging Xenoreactive Antibodies to Alleviate Cocaine-Induced Behavior†

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Abstract

A method for potentiating the response to an anti-cocaine vaccine by leveraging xenoreactive antibodies against the carbohydrate epitope Gala1,3-Gal (GAL) was found to result in a highly specific anti-cocaine response that was able to significantly attenuate cocaine-induced locomotion at 20 mg/kg with superior efficacy compared to a standard conjugate.

Graphical abstract

A bioconjugate targeted to prefomed, xenoreactive antibodies also containing a cocaine hapten elicits a potent and specific anti-cocaine response able to significantly attenuate cocaine-induced behavior.

Cocaine remains a dangerous and prevalent abused substance, with an estimated 18.3 million cocaine users worldwide.¹ Cocaine is the most commonly used illicit stimulant in the EU, with an estimated 2.4 million users aged 15–34.² In the United States, it is estimated there are 1.5 million cocaine users aged 12 and older³, and there has been a 42% increase in the number of deaths attributed to cocaine from 2002 to 2014.⁴ Despite its prevalence, there is still no clinically-approved intervention for cocaine addiction. One strategy has been to attempt to formulate an anti-cocaine vaccine, which would raise an antiserum against the drug, and sequester it in the blood, preventing the intended effects.⁵

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A large portion of vaccine research is centered on the discovery and implementation of adjuvants, or substances that can increase the immunogenicity of a relatively weak or non-immunogenic antigen (Ag). Many adjuvants act broadly as immunostimulants (such as CpG and MPLA), agonizing pleiotropic pathways to prime the immune response for the vaccine, while others serve as vehicles for delivery of antigen in a specific manner to affect potentiation.⁶ Despite rapid progress in discovery, many adjuvants have struggled to be clinically implemented due to undesirable side effects; it remains unknown if these adverse effects can be dissociated from the intended effect.^{6,7} While B-cells are the primary mediator of the Ag-specific antibody response, it has been established that the interaction of helper T-cells with Ag-non-specific antigen-presenting cells (APC), such as macrophages or dendritic cells, is also necessary for an efficient response.⁸ There is also evidence that immunostimulatory adjuvants act to potentiate vaccines primarily through affecting Ag-non-specific APC.⁹ One method to adjuvant a vaccine formulation that has arisen is via exploiting the Fc γ receptor (Fc γ R) to enhance antigen uptake and presentation by APC via complex formation with the antibody (Ab) Fc.¹⁰ These complexes stimulate the release of cytokines for the recruitment of effector cells, such as CD4⁺ T-cells.¹¹ It is known that Ag-Ab immunocomplexes can stimulate an increased response if certain criteria are met: 1.) Complex formation occurs *in vivo*.¹² 2.) The Ag is in excess of the Ab.^{12,13} 3.) The Ab of the complex is primarily IgG1 subtype.¹⁰ This has led to a scheme whereby the immune response to a non-immunogenic Ag would be potentiated due to assisted uptake by preformed Abs directed against an epitope either covalently or non-covalently associated to the Ag of interest. This uptake then provides increased presentation by APC to helper T-cells (Figure 1).

Administration of an exogenous Ab directed against a vaccine formulation would prove tedious, especially if the Ab is directed against the epitope of interest. Naturally-occurring preformed Abs, often posing a barrier in xenotransplantation,^{14,15} have been exploited in attempts to leverage the effector functions of this repertoire in order to direct an immune response against tumor antigens and infectious disease. Thus, leveraging a preformed, natural Ab is desirable. Of the natural antibody repertoire, those directed against the carbohydrate Ag Gal α 1-3Gal (GAL, **8**) are one of the most abundant, comprising about 1% of the IgG in normal human serum.^{16–18} Initial studies leveraging this natural Ab reservoir centered on utilizing the GAL epitope to increase the efficacy of immunization against HIV gp120 and peptides of influenza virus.^{19,20} Subsequently, liposomal formulations of peptide vaccines were also used.²¹ We endeavored to exploit these preformed antibodies to increase the efficacy of an anti-cocaine vaccine, but it was unknown whether this method would provide an increased response to a small-molecule. Another natural reservoir that has been explored in this manner is against L-rhamnose, however it appears these Abs are of lower affinity to the single saccharide epitope.^{22,23} We tested this plan of action utilizing the GNE hapten, **5**, to determine if this strategy would potentiate the response to the vaccine or if the presence of the GAL epitope would potentially shield the GNE epitope from a response.

In order to accomplish this, we used a conjugation tactic that would allow us to create a T-cell epitope carrier protein functionalized with both GNE and GAL haptens (Scheme 1). Many strategies have been developed to leverage the natural anti-GAL Ab reservoir,

utilizing liposomes²¹ and ghosts developed from rabbit red blood cells (RRBC).¹⁵ These methods often involve multiple components, complicating the formulation, and resulting in heterogeneous mixtures. Our chemical strategy results in a more homogeneous and easily characterized formulation. The GAL epitope is a type-2 terminus most commonly capping glucosamine residues,^{21,24} and it has been established that the preferred target antigen of the natural anti-GAL repertoire is directed against the trisaccharide Gal α 1-3Gal β 1-4GlcNAc, which provides a decreased conformational flexibility of the epitope.^{25–27} Thus, the GAL hapten (**1**) was produced in high quantity using a two-component chemoenzymatic process resulting in the GAL trisaccharide with an azide handle appended.^{28,29} This substrate can then undergo reduction to the amine, followed by coupling to a squaric acid moiety to produce the GAL hapten **1**. Hapten **1** is coupled to a T-cell epitope carrier, **2**, via incubation in borate buffer to react with free lysine residues (Scheme 1).³⁰ This coupling reaction resulted in 1 or 2 copies of the **1** on the carrier protein (Supplementary Figure 1). Importantly, this conjugation strategy leaves unreacted lysine residues intact, allowing for a subsequent reaction of the GNE hapten,³¹ **3**, to be conjugated using EDC/sNHS conditions (Scheme 1). It was found that this resulted in 15–20 copies of **3** per carrier by MS analysis (Supplementary Figure 2). The resulting double immunoconjugate of TT (**6**) or OVA (**7**) is then dried for formulation for injection.

Mice were prevaccinated as reported previously.²⁸ All vaccinated groups responded similarly to treatment with **3** (Table 1). It was also found that the non-prevaccinated group had a detectable, albeit low, titer for **1**, but there was no detectable affinity for **1** by SPR (Table 1). It has been reported that the IgG1 subclass is most effective at enhancing antigen presentation.¹⁰ We found that while the formulation with **3** tended to induce a primarily IgG1 response, the naturally arising anti-GAL IgG in the non-vaccinated mice was split between IgG1 and IgG3 (Table 2, Supplementary Figure 5). The distribution of anti-**1** Abs in normal human serum samples were found to be similar to that induced by **3** (Table 2). Although there is a tenuous parallel between murine and human FcR functions, in humans, all subtypes of IgG can act on Fc γ RIIIA.³²

At 58 days, mice were inoculated using the double conjugate formulations using either TT (**6**) or OVA (**7**) as carriers, with or without **1** appended (Figure 2A). Alum was chosen as a well-delineated, weak, Th2 adjuvant that would allow for differences regarding the presence of **1** to be discerned.^{6,7} Mice were given booster injections at 2 and 4 weeks of the same formulation, with bleeds taken 10 days after each boost (Figure 2A). It was found that although little difference existed in the anti-**1** titer or GAL affinity between prevaccinated groups, there was a large difference between groups in the affinity and binding capacity for cocaine (Table 1).

Behavioral testing of cocaine-induced locomotion was undertaken to test the efficacy of the vaccine treatments. In all cases, treatment was able to significantly attenuate hyperlocomotion in response to cocaine at 10 mg/kg (Figure 2C), however, in a subsequent test at 20 mg/kg, all groups lost significant attenuation except the group vaccinated with **7** (Figure 2D). Few active anti-cocaine vaccines have been able to demonstrate efficacy at a single dose this high. Only recently, a vaccine using **5** requiring the powerful immunostimulatory adjuvant CpG was able to significantly attenuate locomotor behavior at

20 mg/kg in a single dose.³³ Most efficacies are only observed after repeated exposure to cocaine, wherein non-vaccinated mice become more sensitized to the psychoactive effects of cocaine.^{31,34} The observed attenuation in response to **7** was in congruence with analysis of the blood:brain distribution of cocaine, which was found to be most sequestered in that group (Figure 2B), although the difference was statistically insignificant.

Unlike an anti-cancer or infectious disease vaccine, it is imperative that an anti-cocaine vaccine produce a robust and tight-binding response.⁵ A radioimmunoassay (RIA) was employed in order to better determine the affinity and binding capacity of the antiserum for cocaine.³⁵ It was found that the group vaccinated with **7** had a lower overall binding capacity, but an affinity for cocaine two orders of magnitude better than the other groups, at 34.2 nM (Table 1). This would explain the ability of this group to best sequester cocaine in the serum and superior performance in the behavioral testing. Thus it would appear the presence of **1** on the immunoconjugate was able to drive a more potent and effective immune response to **5**.

It is notable that groups vaccinated using **6** had significantly lower anti-**5** titers, as well as poorer performance in blocking cocaine-induced locomotion in the behavior tests. We hypothesize the use of **3** to vaccinate the mice against GAL allowed for the presence of a significantly greater number of epitopes of **6** to be bound due to the high degree of similarity. The antibody repertoire raised in response to **3** could mask the presentation of the cocaine epitope of **5**, and previous studies report that potentiation is greatest when there is an excess of Ag.⁶ Thus, it may be imperative a different carrier be used, at least when the second vaccination is so close to the first. The anti-TT titer at day 79 for the non-prevaccinated group was similar to that of day 51 for the other two groups, which indicate this factor may be most important prior to the primary response (Supplementary Figure 4). Unfortunately, we were unable to find another carrier for **1** that could raise a suitable response to perform the inverse experiment (Supplementary Figure 5). Continued efforts will focus on finding an orthogonal carrier to TT to test this hypothesis.

In conclusion, we have demonstrated a synthesis of an immunoconjugate (**7**) containing both a cocaine hapten (**5**) and a GAL epitope (**1**) to leverage preformed anti-**1** antibodies to adjuvant the anti-cocaine response. While all treatments were effective in producing a viable anti-cocaine response, the most dramatic effects were provided by the conjugate with **7**, when the carrier protein was changed from the prevaccination against **1**. Importantly, this strategy resulted in an antibody response with higher affinity for cocaine that was significantly able to attenuate cocaine-induced locomotion at 20 mg/kg. This represents the first demonstration of this strategy in potentiating a vaccine against a small-molecule, significantly broadening its potential.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Notes and references

1. Citations United Nations Office on Drugs and Crime. World Drug Report 2016. United Nations publication, Sales No. E.16.XI.7
2. European Monitoring Centre for Drugs and Drug Addiction. European Drug Report 2016: Trends and Developments. Publications Office of the European Union; Luxembourg: 2016.
3. Center for Behavioral Health Statistics and Quality. Behavioral Health Trends in the United States: Results from the 2014 National Survey on Drug Use and Health. 2015. HHS Publication No. SMA 15-4927, NSDUH Series H-50Retrieved from <http://www.samhsa.gov/data/>
4. National Institute on Drug Abuse. Cocaine. 2016
5. Janda KD, Treweek JB. Nat Rev Immunol. 2012; 12:67–72.
6. Montomoli E, Piccirella S, Khadang B, Mennitto E, Camerini R, De Rosa A. Expert Rev Vaccines. 2011; 10:1053–1061. [PubMed: 21806399]
7. Petrovsky N, Aguilar JC. Immunol Cell Biol. 2004; 82:488–496. [PubMed: 15479434]
8. Mosser DM, Edwards JP. Nat Rev Immunol. 2008; 8:958–969. [PubMed: 19029990]
9. O'Hagan DT, Valiante NM. Nat Rev Drug Discov. 2003; 2:727–735. [PubMed: 12951579]
10. Gosselin EJ, Wardwell K, Gosselin DR, Alter N, Fisher J, Guyre PM. Immunol. 1992; 149:3477–3481.
11. Nimmerjahn F, Ravetch JF. Nat Rev Immunol. 2008; 8:34–47. [PubMed: 18064051]
12. Stoner RD, Terres GJ. Immunol. 1963; 91:761–770.
13. Manca F, Fenoglio D, Li Pira G, Kunkl A, Celada F. J Exp Med. 1991; 173:37–48. [PubMed: 1985125]
14. Cooper DK, Koren E, Oriol R. Immunol Rev. 1994; 141:31–58. [PubMed: 7868156]
15. Cooper DK. Glycobiology. 2016; 26:571–581. [PubMed: 26935763]
16. Galili U, Rachmilewitz EA, Peleg A, Flechner IJ. Exp Med. 1984; 160:1519–1531.
17. Galili U, Anaraki F, Thall A, Hill-Black C, Radic M. Blood. 1993; 82:2485–2493. [PubMed: 7691263]
18. Wang L, Radic MZ, Galili U. J Immunol. 1995; 155:1276–1285. [PubMed: 7543518]
19. Abdel-Motal UM, Wang S, Lu S, Wigglesworth K, Galili U. J Virol. 2006; 80:6943–6951. [PubMed: 16809300]
20. Abdel-Motal UM, Guay HM, Wigglesworth K, Welsh RM, Galili U. J Virol. 2007; 81:9131–41. [PubMed: 17609270]
21. Abdel-Motal UM, Wigglesworth K, Galili U. Vaccine. 2009; 27:3072–3082. [PubMed: 19428921]
22. Sarkar S, Lombardo SA, Herner DN, Talan RS, Wall KA, Sucheck SJ. J Am Chem Soc. 2010; 132:17236–17246. [PubMed: 21080675]
23. Li X, Rao X, Cai L, Liu X, Wang H, Wu W, Zhu C, Chen M, Wang PG, Yi W. ACS Chem Biol. 2016; 11:1205–1209. [PubMed: 26982552]
24. Galili U, Basbaum CB, Shohet SB, Buehler J, Macher BA. J Biol Chem. 1987; 262:4683–4688. [PubMed: 2435715]
25. Galili U, Shohet SB, Kobrin E, Stults CL, Macher BA. J Biol Chem. 1988; 263:17755–17762. [PubMed: 2460463]
26. Cooper DK, Good H, Koren E, Oriol R, Malcolm AJ, Ippolito RM, Neethling FA, Ye Y, Romano E, Zuhdi N. Transplant Immunol. 1993; 1:198–205.
27. Wang JQ, Chen X, Zhang W, Zacharek S, Chen Y, Wang PG. J Am Chem Soc. 1999; 121:8174–8181.
28. Anraku K, Shun S, Jacob NT, Eubanks LM, Ellis BA, Janda KD. Org Biomol Chem. 2017; 15:2979–2992. [PubMed: 28294277]

29. Fang J, Li J, Chen X, Zhang Y, Wang J, Guo Z, Zhang W, Yu L, Brew K, Wang PG. J Am Chem Soc. 1998; 120:6635–6638.
30. Wurm FR, Klok HA. Chem Soc Rev. 2013; 42:8220–18. [PubMed: 23873344]
31. Cai X, Whitfield T, Hixon MS, Grant Y, Koob GF, Janda KD. J Med Chem. 2013; 56:3701–3709. [PubMed: 23627877]
32. Bruhns P. Blood. 2012; 119(24):5640–5649. [PubMed: 22535666]
33. Kimishima A, Wenthur CJ, Eubanks LM, Sato S, Janda KD. Mol Pharm. 2016; 13:3884–3890. [PubMed: 27717287]
34. Koob GF, Hicks MJ, Wee S, Rosenberg JB, De BP, Kaminsky SM, Moreno A, Janda KD, Crystal RG. CNS Neurol Disord Drug Targets. 2011; 10:899–904. [PubMed: 22229312]
35. Müller R. Methods in Enzymology. 1983; 92:589–601. [PubMed: 6190069]

Figure 1. Depiction of the purported mechanism for leveraging preformed anti-GAL antibodies to potentiate an anti-cocaine vaccine.

Figure 2.

(A) Vaccination schedule. Numbers indicate days post-GAL-TT priming. (B) Blood-brain barrier distribution ratio for each group. No significant difference was found between groups. (C) Cocaine-induced locomotion at 10 mg/kg ($F(102,493)=3.22$) and (D) 20 mg/kg cocaine ($F(102,476)=1.94$). Dotted line indicates time of cocaine administration. * $p<0.05$, ** $P<0.01$, *** $p<0.001$.

Scheme 1.
Synthesis of immunoconjugates.

Table 1

Average serum specificity for cocaine.

Group	Anti-1 titer at Bleed 1	1 K_i (μ M) ^a	Cocaine K_D (nM) ^b	Ab (μ M) ^b
6	49478 $\pm$ 6056	1.78 $\pm$ 0.10	4790 ^c	2.0 $\times$ 10 ⁻⁶ ^c
5 – TT	72253 $\pm$ 8394	1.06 $\pm$ 0.12	637 ^c	5.7 $\times$ 10 ⁻⁷ ^c
5 – OVA	57377 $\pm$ 11229	1.58 $\pm$ 0.12	1005 $\pm$ 364	1.1 $\pm$ 0.023 $\times$ 10 ⁻⁶
7	61513 $\pm$ 15711	0.88 $\pm$ 0.12	34.2 $\pm$ 13.7	2.0 $\pm$ 0.016 $\times$ 10 ⁻⁸
6 (no prevax)	230 $\pm$ 88	N.D. ^d	1240 $\pm$ 210	7.5 $\pm$ 0.23 $\times$ 10 ⁻⁷

^aDetermined by competitive SPR (see supplement).^bDetermined by RIA.^cOnly one measurement could be taken, due to high serum concentration needed.^dN.D. = not detectable.

Table 2End-point ELISA for IgG Subtypes.^a

Subtype:	IgG1	IgG3
6	2.3 ± 0.15	0.15 ± 0.03
5 – TT	2.3 ± 0.17	0.09 ± 0.01
5 – OVA	2.2 ± 0.16	0.11 ± 0.02
7	2.1 ± 0.20	0.09 ± 0.01
6 (no prevax)	1.4 ± 0.17	0.56 ± 0.24
Human Serum ^b	1.9 ± 0.39 ^c	0.41 ± 0.12 ^c

^a OD values at 452 nm.^b n = 10.^c using anti-hu secondary Ab.