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A human recombinant monoclonal antibody to cocaine: Preparation, characterization and behavioral studies

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Abstract

Cocaine abuse remains prevalent worldwide and continues to be a major health concern; nonetheless, there is no effective therapy. Immunopharmacotherapy has emerged as a promising treatment strategy by which anti-cocaine antibodies bind to the drug blunting its effects. Previous passive immunization studies using our human monoclonal antibody, GNCgzk, resulted in protection against cocaine overdose and acute toxicity. To further realize the clinical potential of this antibody, a recombinant IgG form of the antibody has been produced in mammalian cells. This antibody displayed a high binding affinity for cocaine (low nanomolar) in line with the superior attributes of the GNCgzk antibody and reduced cocaine-induced ataxia in a cocaine overdose model.

Keywords

Cocaine; Overdose; Immunopharmacotherapy; Passive Immunization; Monoclonal Antibody

Cocaine is a powerfully addictive stimulant drug that induces subjective effects and euphoria by binding to the dopamine transporter in the brain resulting in reuptake inhibition, accumulation of extracellular dopamine, and an increase in dopaminergic neurotransmission (Figure 1).¹ Cocaine dependence and the potential for drug overdose can be attributed to the

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Supplementary Material

Supplementary material associated with this article can be found, in the online version.

pleasure and rewarding effects induced by the elevated synaptic levels of dopamine in the brain. According to the 2012 National Survey on Drug Use and Health (NSDUH), an estimated 1.6 million Americans aged 12 and older are cocaine users with approximately 1.1 million meeting the clinical criteria for dependence or abuse of this illicit drug.² Additionally, a number of serious health conditions may result from cocaine use such as psychosis, cardiac arrest, strokes, seizures, and even mortality; therefore, cocaine dependence remains to be a major public health concern. Cocaine users are also at higher risk for contracting HIV and hepatitis due to their overall lifestyle and drug-influenced risky behavior.

Despite many years of research into the treatment of cocaine abuse, no medication has emerged as consistently effective for the treatment of cocaine dependence or acute toxicity; in fact, no drug has been approved by the Food and Drug Administration (FDA) in the United States or by similar agencies in other countries. Progress in understanding the neurobiology of how cocaine affects the brain has led to the discovery of a number of pharmacotherapeutic treatments that modulate dopamine receptors, different neurotransmitter systems, and neurological processes.³ However, all of these medications target the same neurocircuitry in the brain affected by cocaine and not the actual drug molecule itself.

Immunopharmacotherapy provides two alternative strategies: 1) active vaccination which stimulates the immune system to produce endogenous anti-cocaine antibodies and 2) passive immunization which relies on the administration of exogenous monoclonal antibodies (mAbs) against cocaine. The overall goal of this approach is for the cocaine specific antibodies to bind enough free circulating cocaine in the periphery keeping the cocaine levels in the brain below its minimally effective concentration, as well as reduce both central nervous system and peripherally mediated adverse effects caused by the drug.⁴

Our laboratory recently disclosed the discovery of a fully human anti-cocaine mAb, GNCgzk, produced from a transgenic mouse (Xenomouse) which has a 10-fold improvement in cocaine binding affinity with respect to our previously reported murine antibody GNC92H2; GNCgzk also was superior in protecting from cocaine-induced toxicity.⁵ The high binding affinity ($K_d = 0.18$ nM), great specificity, and fast association rate ($k_{on} = 4.3 \times 10^7$ M⁻¹ s⁻¹) of GNCgzk are key elements in its effectiveness. The IgG - gzk prevented lethality in all immunized mice in a prophylactic scenario where mAb was administered 30 min prior to cocaine injection. Additionally, there was a significant lowering of animal deaths to 20% using a posttreatment overdose model in which the antibody was administered 3 min after cocaine injection. An alternative GNCgzk antibody format, F(ab')₂, also provided protection against lethality post-treatment, as well as reduced the severity of ataxia and seizures. It is important to note that in all of these studies there is a large molar excess of cocaine with respect to antibody. Overall, the affinity, pharmacokinetic and pharmacodynamic attributes, and its ability to reverse cocaine toxicity establishes the GNCgzk antibody as one of the most important cocaine neutralizing antibodies reported to date.

Based on the highly positive results with the GNCgzk antibody, we decided to move forward with this antibody and produce a recombinant monoclonal form of the antibody in mammalian cells, thereby allowing for further studies. To achieve this goal, the light and heavy chain variable regions, V_L and V_H , of the GNCgzk antibody were amplified from the cDNA and cloned into a mammalian expression vector containing the human IgG.1 constant heavy chain (C_H : C_{H1} , C_{H2} , and C_{H3}) and constant kappa light chain (C_K) genes illustrated in Figure 2. The expression vector also contained copies of the dhfr gene encoding dihydrofolate reductase allowing for transfection, selection, and expression of the recombinant human IgG-gzk protein in dhfrdeficient CHO-DG44 cells.⁶ After expression and purification of a IgG-gzk mAb, the binding affinity of the antibody for cocaine was analyzed by equilibrium dialysis (radioimmunoassay) and determined to be $K_d = 0.4$ nM.⁷ The tight binding of our newly produced IgG antibody is in accordance with the IgG mAb previously produced from the transgenic mouse.

To establish a baseline for our recombinant IgG-gzk antibody, we tested its efficacy in mice using our post-treatment cocaine overdose model which evaluates the mAb's ability to reverse a potentially lethal overdose after the appearance of outward signs of acute toxicity. Briefly, an LD₅₀ dose of cocaine (93 mg/kg) was administered (ip) followed by an antibody dose of 180 mg/kg (iv) 3 min after cocaine exposure and the behavioral response to cocaine toxicity was observed for 60 min. In this particular experiment, the previously published LD₅₀ dose of cocaine (93 mg/kg) did not result in the incidence of any animal deaths in neither the cocaine alone group nor the cocaine plus antibody group. The incidence of seizures also did not differ, and were well below that observed in previous publications. There was sufficient cocaine effect to observe an array of premorbid behaviors; however, there were no significant differences in the total premorbid observations based on treatment [Control: 5.29 ± 0.36 , IgG-gzk: 4.78 ± 0.28 , $p = 0.27$]. Upon examining the level of cocaine-induced ataxia, there was an overall significant effect of treatment [$F_{1, 24} = 5.8$, $p < 0.05$], and also a significant interaction between treatment and time post-cocaine [$F_{3, 24} = 2.6$, $p < 0.05$]. Post-hoc analysis demonstrated significantly reduced ataxia scores at various times between 6-24 min after cocaine administration (Figure 3). A repeated pilot test study with the same batch of cocaine in a group of naïve mice ($n = 6$) showed 100 % lethality at 100 mg/kg of cocaine.

While the full therapeutic efficacy of the recombinant IgG-gzk cocaine mAb treatment is unclear due to the insufficient cocaine overdosing to produce full seizure and lethal consequences, the GNCgzk group did show significant reductions in cocaine overdose-induced ataxia. While previously published results did not show reductions in ataxia with similar mAb treatment, it should be noted that the reported ataxia was only measured in the subset of mice that did not succumb to cocaine overdose. Since no subjects in the current study presented with severe seizures or an ultimately lethal outcome, ataxia became the predominate measure of cocaine activity.

Immunopharmacotherapeutic passive vaccination strategies have shown potential as effective antidotal treatments for cocaine overdose. A decrease or abrogation of lethality and the dampening of premorbid symptoms have been achieved in our mAb studies despite the administration of > 100-fold molar excess of cocaine compared to antibody binding sites.⁵

While we can hypothesize as to the mechanism of this effect based on previous outcomes, complementary studies may provide a deeper understanding of this phenomenon. Our mAb GNC-gzk has proven to be a superior candidate for further investigative studies due to its high affinity, specificity, and overall potency; therefore, we undertook the task of producing a human recombinant form of the IgG-gzk antibody in mammalian cells. Our recombinant IgG-gzk displayed similar binding affinity as compared to the original IgG-gzk from the transgenic mouse. Interestingly, the mAb provided a reduction in cocaine-induced ataxia, when tested in our cocaine overdose model under the conditions described *vide supra*. In sum, this recombinant version of IgG-gzk will provide us the means to continue evaluating the clinical potential of GNCgzk based therapeutics for the treatment of cocaine-induced acute toxicity and lethality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Chemical structure of the stimulant drug cocaine.

Figure 2.

GNCgzk human IgG γ_1 mammalian expression vector constructed for production of a monoclonal antibody in dhfr-deficient CHO-DG44 cells.

Figure 3.

Premorbid ataxia scores based on observations every 3 min post-cocaine administration, in mice treated with either control or IgG-gzk monoclonal antibody treatment. * $p < 0.05$, ** $p < 0.01$ difference between treatment groups based on corrected factorial nonparametric post-hoc testing, $n = 7-9$ mice/treatment.