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An Antidote for Acute Cocaine Toxicity

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Abstract

Not only has immunopharmacotherapy grown into a field that addresses the abuse of numerous illicit substances, but also the treatment methodologies within immunopharmacotherapy have expanded from traditional active vaccination to passive immunization with anti-drug monoclonal antibodies, optimized mAb formats, and catalytic drug-degrading antibodies. Many laboratories have focused on transitioning distinct immunopharmacotherapeutics to clinical evaluation, but with respect to the indication of cocaine abuse, only the active vaccine TA-CD, which is modeled after our original cocaine hapten GNC¹, has been carried through to human clinical trials.² The successful application of murine mAb GNC92H2 to the reversal of cocaine overdose in a mouse model prompted investigations of human immunoglobulins with the clinical potential to serve as cocaine antidotes. We now report the therapeutic utility of a superior clone, human mAb GNCgzk ($K_d = 0.18$ nM), which offers a 10-fold improvement in cocaine binding affinity. The GNCgzk manifold was engineered for rapid cocaine clearance, and administration of the F(ab')₂ and Fab formats even after the appearance of acute behavioral signs of cocaine toxicity granted nearly complete prevention of lethality. Thus, contrary to the immunopharmacotherapeutic treatment of drug self-administration, minimal antibody doses were shown to counteract the lethality of a molar excess of circulating cocaine. Passive vaccination with drug-specific antibodies represents a viable treatment strategy for the human condition of cocaine overdose.

Keywords

Cocaine; Drug abuse; Overdose; Immunopharmacotherapy; Antidote; Vaccine; Passive immunization; Monoclonal antibody; Mice

INTRODUCTION

Despite ongoing research into the treatment of cocaine abuse, effective medications for acute toxicity or chronic addiction are still lacking. Cocaine functions as a molecular blocker of dopamine transporters, and thus drug detoxification within the CNS via its displacement from transporter binding sites poses an interesting conundrum. We hypothesized that an immunopharmacotherapeutic treatment strategy for cocaine-related toxicity would promote drug efflux from the CNS through binding cocaine in the periphery. Cocaine vaccines circumvent the prerequisite of a central mechanism to effect cocaine trafficking, a barrier

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SUPPORTING INFORMATION AVAILABLE

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that has proven insurmountable to other pharmacotherapeutic approaches. Herein, active and passive vaccination strategies that yield therapeutic levels of cocaine-binding antibodies have been explored in rodent models for drug detoxification as well as for the prevention of drug acquisition, self-administration, and reinstatement.^{3–8} One of these vaccine candidates, termed TA-CD, has entered phase III clinical trials for addiction management, but overall, the rodent and human studies have met varying degrees of success.^{2,9–11}

Nevertheless, the advancement of three nicotine vaccines and a cocaine vaccine through phase I and/or phase II clinical trials has established immunopharmacotherapy as a viable treatment strategy that will become medically relevant after further improvements in efficacy.¹²² A major obstacle to their approval for medical use has been the high variability of antibody titers in vaccinated human participants, which correlated to the mixed efficacy of each vaccine candidate in increasing the continuous abstinence rate. With respect to TA-CD, this shortcoming may relate to the poor adjuvanticity of its cholera toxin b carrier protein, an outdated choice but whose clinically safe reputation in vaccine formulations trumps its modest efficacy.^{2,9,13} It follows that the more recent advances in the field of vaccine design have yet to generate a practical anti-cocaine immunization strategy.¹⁴ During the last decade, our research in immunopharmacotherapy has validated the ability of our GNC hapten to elicit a robust polyclonal response whose antibodies possess consistently high affinity and specificity for cocaine.^{1,6,15} This was illustrated by the isolation and characterization of the murine clone GNC92H2, whose superior cocaine-binding properties matched its therapeutic utility in rodent models of cocaine reinstatement and overdose prevention.^{5,7,16}

To serve as an antidote to the potentially lethal effects of acute cocaine toxicity, we hypothesized that a human or humanized antibody of higher affinity than murine mAb GNC92H2 ($K_d = 2$ nM) would be required. Given that IVIg infusions are delivered to achieve > 5 g/L serum IgG concentrations in humans, we speculated that micromolar levels of anti-cocaine IgG (20 μ M cocaine binding capacity in serum, or ~ 1.5 g IgG per liter blood volume) would be clinically attainable, and a nanomolar K_d could bind a sufficient level of circulating cocaine to drive partitioning from brain into peripheral circulation or antibody-bound state and potentially reverse cocaine-induced symptomology (e.g., seizures, cardiotoxicity, death). To lend clinical value to our studies focusing on cocaine antidotes, it was tantamount to identify the minimum antibody dose required to confer a therapeutic effect given the medical challenge, potential toxicity, and expense of administering a high protein dose to humans. The relationship between GNC92H2 format dose and its corresponding prevention of overdose symptoms was briefly investigated, and it was found that lowering the F(ab')₂-92H2 dose from 132 mg/kg to 66 mg/kg did not lead to a corresponding drop in its therapeutic ability. The rate of lethality remained at 20% for both dose groups, and the differences in premorbid behaviors and seizure generation between these two dosing groups were statistically indistinguishable by the χ^2 test of independence (132 mg/kg vs. 66 mg/kg: ataxia, $\chi^2 = 7.1$, $p > 0.2$; seizures, $\chi^2 = 2.2$, $p > 0.50$).

Thus, the current study represents our more intensive efforts to evaluate clinically relevant anti-cocaine passive vaccines in an established animal model of acute cocaine toxicity. Through an alliance with Abgenix Biopharma, now Amgen Inc., we gained access to the XenoMouse®, a transgenic mouse that produces fully human antibodies when challenged with antigen (see Supporting Information).^{17,18} Based on the previous success of our cocaine-like haptens GNC¹ and GND⁶, XenoMouse immunizations were conducted with the GNC-KLH immunogen, and the fully human anti-cocaine mAb, termed GNCgzk was obtained after initial screens of 1,553 anti-GNC binders and advanced cloning of three mAbs with high affinity for cocaine. Pharmacokinetic analysis of the final six unique anti-GNC recombinant antibodies alongside mAb GNC92H2 and other mAbs reported in the

literature permitted an accurate comparison of their K_d values (see Supporting Information). Importantly, mAb GNCgzk ($K_d = 0.18$ nM) exhibited a 10-fold improvement in affinity for cocaine over murine mAb GNC92H2 ($K_d = 2.0$ nM); GNCgzk had little affinity to the metabolites benzoylecognine and ecognine methyl ester (0.4 μ M and 79 μ M respectively, while it bound potently to cocaethylene (0.5 nM).

Given the superior cocaine affinity of GNCgzk relative to GNC92H2 and other anti-cocaine mAbs reported in the literature, it is not anticipated that further improvement of its pharmacodynamic properties is easily attainable or would greatly enhance its therapeutic utility. A preclinical validation of GNCgzk for the treatment of indications related to cocaine abuse would grant its subsequent clinical testing without the need for humanization. Herein, this study depicts the synthetic generation of the IgG, F(ab')₂, and Fab formats of mAb GNCgzk for evaluation in a murine model of cocaine toxicity and their comparison to the corresponding GNC92H2-based vaccines¹⁶ as a method for critically assessing their preclinical merit.

EXPERIMENTAL

We have previously engaged in the expression and purification of fully human mAbs against small-molecule targets, and so the production of GNCgzk IgG, F(ab')₂, and Fab followed the established methods of our laboratory. The scFv of GNCgzk was constructed via cloning and amplification of the variable region genes (V_L and V_H) from the cDNA of the original GNCgzk IgG2 γ and addition of a (GGGS)₃ linker. The cloned variable regions were carried through to the construction of the GNCgzk Fab and IgG, described below, while the F(ab')₂ format was obtained via pepsin digestion of the IgG-gzk.

Production and Purification of Anti-cocaine mAb GNCgzk Fab

The Fab-gzk format was obtained via PCR assembly of the cloned variable region genes (V_H and $V_L\kappa$) of mAb GNCgzk with the heavy (C_{H1}) and light ($C_L\kappa$) chain constant region genes for a human IgG γ_2 . In brief, the heavy chain variable region gene (V_H) and light chain variable region gene ($V_L\kappa$) were amplified directly from the GNCgzk pcDNA. The constant region genes of the antibody heavy chain (C_{H1}) and light chain ($C_L\kappa$) were amplified from our Fab expression vector (pETFlag), which has been used extensively by our laboratory in the synthetic production of human antibodies, including the human-mouse chimera of the anti-cocaine GNC92H2 Fab.^{16,19,20} V_H and C_{H1} fragments were assembled to form the fd gene, and $V_L\kappa$ and $C_L\kappa$ fragments were assembled into the κ light chain. Overlap PCR was used to assemble the full-length Fab-gzk from the heavy (V_H - C_{H1}) and light ($V_L\kappa$ - $C_L\kappa$) chain genes. Engineered to possess flanking Sfi I sites, the PCR-amplified Fab-gzk gene (primers HVHSfi and HJLSfi) was digested with Sfi I (Roche), agarose gel purified, and cloned into the Sfi I digested pETHis expression vector. This vector permits the appendage of an omp A leader sequence to the N-terminus of the light-chain peptide as well as a hexahistidine sequence (His-tag) to the C-terminus for subsequent purification of the protein product. In combination with the pELB leader sequence joined to the N-terminus of the heavy chain peptide, the leader sequences direct the translocation of the polypeptide chains into the bacterial host periplasm for the protein folding and disulphide bond formation required to form functional Fab proteins. The plasmid containing the Fab gene was transformed into *E. coli* DH5 α cells for confirmation of the Fab-gzk product via DNA sequencing. For overproduction, the purified Fab-gzk DNA was transformed into *E. coli* BCL21 cells, and cultures were grown at 37 °C in modified super broth (48 mM 3-morpholin-4-ylpropane-1-sulfonic acid (MOPS, Free Acid), 3% w/v tryptone, 2% w/v yeast) supplemented with 100 μ g/ml carbenicillin until an OD₆₀₀ ~ 0.6–0.8 was reached. Fab-gzk expression was induced by the addition of 1.0 mM isopropyl- β -D-thio-galactoside (IPTG), and cultures were incubated at 30 °C overnight. The His-tagged Fab-gzk was then extracted

and purified via Ni^{2+} -NTA affinity chromatography and, when necessary, Protein-G Sepharose chromatography. UV spectroscopy and SDS-PAGE gel chromatography were implemented to monitor Fab protein concentration and purity, respectively, before additional steps were undertaken to concentrate and purify the protein in preparation for *in vivo* testing.

Enzyme-linked Immunosorbent Assay

Cocaine-binding activity of the synthetically prepared GNCgzk Fab format was compared to that of the IgG standard by ELISA. The cocaine hapten GNC was coupled to bovine serum albumin (BSA), and the GNC-BSA conjugate was applied to an ELISA plate (CoStar; 96-well, half-volume) at a concentration of 10 $\mu\text{g}/\text{ml}$ in PBS at 37 °C for 1 h with shaking, with unmodified BSA serving as a negative control. After washing with distilled water ten times, the wells were blocked for 1 h at 37 °C with 50 μL of Blotto (5% QuikBlot powder in PBS). Aliquots (25 μL total volume, in Blotto) of Fab protein samples, which were collected throughout its production (e.g., after expression, concentration, purification steps), were added to the first row and serially diluted down the plate. A quantification standard of mAb GNCgzk dilution stock (IgG-gzk and/or F(ab')_2 -gzk) covering a range of concentrations (0.005 to 5.0 $\mu\text{g}/\text{mL}$ per well) was also plated in a column alongside Fab samples, and plates were incubated for 1.5 h at 37 °C. After washing, 25 μL of a 1:5,000 dilution of a goat-anti-mouse IgG (heavy and light chain) horseradish peroxidase conjugate (Thermo Fisher Scientific Inc.; Waltham, MA), goat-anti-mouse IgG (Fab-specific) horseradish peroxidase conjugate (Sigma), or His-probe (H-3) horseradish peroxidase conjugate (mouse monoclonal IgG; Santa Cruz Biotechnology Inc., Santa Cruz, CA) in Blotto was added to wells for a 1-h incubation at 37 °C. The plate was developed with the colorimetric reagent tetramethylbenzidine substrate (TMB, 50 $\mu\text{L}/\text{well}$, Pierce), quenched with an equal volume of 2 M H_2SO_4 , and the absorbance at 450 nm measured on a 96-well ELISA plate reader.

Production and purification of anti-cocaine mAb GNCgzk F(ab')_2

The bivalent F(ab')_2 fragment was generated via pepsin digestion of the purified IgG-gzk stock. To determine the optimal reaction conditions, pilot digestions were performed in 0.2 M acetate buffer, pH 4 and 4.5, and aliquots were removed at multiple time points for monitoring of digestion progress. The reaction was terminated through the addition of 2 M Tris base, followed by dialysis, Protein A chromatography, and ion-exchange chromatography. Once obtained, the isolated F(ab')_2 -gzk was concentrated on a microdialysis/concentration unit (Amicon Corp.; Danvers, MA) with final protein concentrations measured via spectrophotometry. Extensive endotoxin removal purification steps were undertaken (BioVintage; purity confirmed with Limulus amoebocyte lysate (LAL) testing) before its application to animal studies.

Preparation of GNCgzk formats for *in vivo* studies

After isolation by Ni^{2+} -NTA affinity and/or Protein-G Sepharose chromatography, antibody solutions were buffer-exchanged into PBS. After any *in vitro* characterization of the binding properties of the different formats, the antibody stocks were carried through rigorous endotoxin removal steps (EndocleanTM, Biovintage), equilibrium dialysis, and residual endotoxin detection via the limulus amoebocyte lysate (LAL) assay. The purified antibody was lyophilized for long-term storage at 4 °C or reconstituted in isotonic sterile saline to a concentration appropriate for i.v. administration.

Subjects

Male CD-1 mice (30 $\pm$ 5 g, n $\geq$ 10 for overdose experiments) purchased from Charles River Laboratories (Wilmington, MA) were used. Animals were housed four per cage prior to catheter implantation in a 12:12-h light-dark cycle (lights off at 09:00 h). Water and food

pellets were available *ad libitum* in their living cages. All the experiments described in this study were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the U.S. National Institutes of Health, and were approved by the Animal Care and Use Committee at The Scripps Research Institute. Every effort was made to reduce the number of animals used.

Catheter Implantation

One week after arrival, animals were labeled, weighed, and subjected to intravenous catheterization. Methods for the preparation and surgical implantation of jugular vein catheters in mice are described in detail *vide supra*²¹. Briefly, anesthetized mice were implanted with an indwelling catheter (silastic tubing, id 0.3 mm, od 0.64 mm; 12 mm in length) in the right external jugular. The patency of catheters was maintained via daily infusions of heparinized saline (20 U/flush), and animals were housed singly during the post-operative recovery period and through the course of behavioral testing.

Cocaine Treatment

Animals were administered a 50%-lethal dose ($LD_{50} = 93$ mg/kg) of (–) cocaine hydrochloride (NIDA; Rockville, MD), which was dissolved in sterile 0.9% saline for i.p. injection at a volume of 10 ml/kg.²² Upon injection of the LD_{50} cocaine dose, subjects were transferred to individual locomotor activity cages for observation.

Passive immunization

The mAb-based agent or vehicle (sterile 0.9% saline) was administered via a bolus infusion through a 10-in.-long polyethylene tube attached to the catheter. This permitted the experimenter to deliver the mAb infusion during active recording of subject in the locomotor activity chamber without disrupting its behavior or full ambulatory range. The 30-min mAb pretreatment time point allowed the subject to recover from any stress related to the administration of a large bolus mAb infusion prior to cocaine injection. The 3-min post-cocaine treatment time point was chosen on the basis of previous work where the average onset of seizures, one predictor of lethality, occurred 3.4 minutes after drug injection.⁵ The doses of $F(ab')_2$ (66 mg/kg) and Fab (120 mg/kg and 60 mg/kg) formats, which were adjusted for format valency, were selected to maintain equivalent binding capacities for cocaine across low (1.2×10^{-6} mol cocaine per kg antibody) and high (2.4×10^{-6} mol/kg) mAb dosing schedules. In the 3 min post-cocaine treatment experiment, the lower mAb format dose (e.g., Fab = 60 mg/kg) translates to $< 1/100^{\text{th}}$ the molar amount of cocaine administered in the LD_{50} dose (93 mg/kg), while the higher mAb dose (e.g., Fab = 120 mg/kg) translates to $\sim 1/50^{\text{th}}$ mol Eq of cocaine.

Experiment 1: Prophylactic immunopharmacotherapy to prevent of cocaine-related toxicity—The purpose of this experiment was to establish a benchmark of GNCgzk efficacy in averting the onset of severe symptoms of cocaine toxicity. Its results would be relevant to comparisons with the prophylactic efficacy of murine mAb GNC92H2¹⁶ and with the therapeutic capacity of GNCgzk in antidotal immunization experiments. To summarize the experimental protocol, IgG-gzk was tested at the lower dose level (IgG: 90 mg/kg) in the murine cocaine overdose model that has been described previously.¹⁶ Thirty minutes following i.v. infusion of IgG-gzk or a vehicle saline solution, the LD_{50} cocaine dose was administered by i.p. injection, and test subjects were placed directly into locomotor activity cages for activity monitoring across the entire progression of cocaine-related symptoms. The smaller mAb fragments were not tested in this pretreatment model because their rapid elimination would directly counteract their application to prophylactic immunopharmacotherapy. Conversely, the 30-minute delay between

immunization with IgG-gzk and cocaine injection granted a thorough biodistribution of this mAb format prior to drug exposure, and thus this experiment was expected to reflect the maximum efficacy of an IgG-based therapeutic.

Experiment 2: Immunopharmacotherapeutic antidote to cocaine overdose—To evaluate the different capacity of each mAb GNCgzk format to rescue mice from lethal overdose, subjects were immediately placed in individual locomotor activity cages after cocaine injection (i.p). Attached to the indwelling catheter, a 10 inch-long polyethylene tube permitted the passive vaccine to be administered (i.v.) 3 min after cocaine exposure without interfering with subject behavior. The behavioral response to cocaine toxicity was recorded in the same manner as experiment 1, with all outward signs of acute toxicity recorded for a 60-min period following cocaine injection. Antibody formats were tested at the following doses: 180 mg/kg IgG-gzk, 66 mg/kg F(ab')₂-92H2, 66 mg/kg F(ab')₂-gzk, 60 mg/kg and 120 mg/kg Fab-gzk.

Behavioral monitoring

Behavioral monitoring was conducted via the use of Plexiglas locomotor activity cages (42 × 22 × 20 cm). All mice underwent a brief cage habituation session 24 h prior to behavioral testing. On the test day, mice received an intraperitoneal injection (i.p.) of cocaine and then were immediately transferred to individual cages for a 60-min period of observation. Subject behavior was recorded by a “blind” observer for the time-of-onset, severity, and duration premorbid behaviors (e.g., hyperlocomotion, ataxia, Straub tail response, writhing, convulsions, or loss of righting posture for at least 5 s and/or clonic movements, agitation), seizures (e.g., tonic, clonic, wild-running clonus, tonic-clonic), and death. Specifically, each mouse received a binary score of present (1) or absent (0) for each symptomology (i.e., premorbid behavior, seizures, death) so as to permit the pooling of nominal data by treatment group and the comparison of behavioral observations between groups using the χ^2 test. From these data, the incidence of each overdose symptom within a treatment group was computed as a percentage for ease of depiction in figures.

In addition, the severity of premorbid behaviors and of seizure activity was ranked within successive 3-min time intervals during the 60-min observation period. A scale developed by Sturgeon et al. was applied to the scoring of premorbid ataxia in mice (score 0: inactive; score 1 = unusual, awkward, and/or jerky movements with only occasional loss of balance; score 2 = awkward and jerky movements accompanied by a moderate rate of falling onto their sides but not onto their backs; score 3 = significant loss of balance and falling onto their sides and/or backs when attempting to move about the cage; score 4 = loss of righting ability, or severe impairment of the anti-gravitational reflex to the extent that mice are unable to move beyond a small area within the cage; score 5 = absence of overall movement except for twitching, head bobbing or writhing, and body rolling).²³ The ataxia scores of mice in each treatment group were averaged (mean ± S.E.M.) within each time interval.

A similar ranking system was created for the assessment of seizure severity. Subjects were scored across successive 3-min intervals after cocaine injection based on the following scale: score 0 = absence of seizures; score 1 = short-lasting, mild seizures occasionally accompanied by loss of the righting reflex and with repeated bouts of convulsive activity absent or infrequent; score 2 = convulsive seizures accompanied by severe clonus (wild-running clonus) or rearing, and/or bouts of seizure trains that occur repeatedly or that cause fatal respiratory disorders given their violent nature. These ataxia and seizure ranking systems permitted the severity of overdose symptomology within all mAb treatment groups and the cocaine-alone control group to be compared by analysis of variance (ANOVA),

repeated measures ANOVA, and Fisher's PLSD, or by the χ^2 distribution for nominal data (e.g., the appearance of cocaine overdose symptomology).

Statistical Analysis

To assess the effect of mAb format treatment on cocaine-induced toxicity, the appearance of each overdose symptomology (premorbid behaviors, seizures, death) was assigned a value of incidence (present = 1, absent = 0) for each mouse regardless of the time of onset, and group data were analyzed via the χ^2 distribution ($p < 0.05$). The theoretical frequencies for premorbid behaviors, seizures, and death were based on the cocaine-alone group data. Whereas the χ^2 test of goodness-of-fit was employed to examine the therapeutic benefit of mAb treatment, the χ^2 test of independence or the Fisher's exact test was used to measure differences between immunopharmacotherapeutic treatments in their capacity to alter cocaine-induced symptomology. Lastly, ataxia and seizure severity was scored within successive 3-min time intervals, in which the start of behavioral recording coincided with cocaine injection, and group mean scores (mean $\pm$ S.E.M.) within these intervals were analyzed for parametricity (normal distribution, homogeneity of variance). If these conditions were met, significant differences were determined by analysis of variance (ANOVA) and post-hoc Student's t tests and Fisher's PLSD. In addition, both the maximum ataxia score and the maximum seizure score that were attained within the 60-min session were identified for each subject, and group means for these scores were compared via the statistical tests listed above.

RESULTS

Preliminary comparison of GNC92H2 and GNCgzk-based immunization strategies

Several anti-cocaine passive vaccines, including the murine mAb GNC92H2 (see Fig. 1, IgG-92H2) have been demonstrated to attenuate the appearance of cocaine-induced behaviors *in vivo* when administered prior to drug exposure.¹⁶ The human mAb GNCgzk IgG was evaluated in a mouse model of mAb-based prophylaxis against cocaine overdose, wherein its administration (IgG-gzk, 90 mg/kg, i.v.) 30 min before injection of an LD₅₀ drug dose (cocaine, 93 mg/kg, i.p.) prevented death in all immunized mice ($\chi^2 = 6.087$, $p < 0.014$). Pretreatment with IgG-gzk also ameliorated the acute symptoms of cocaine-related toxicity (Fig. 1).

For the testing of GNCgzk-based vaccines as antidotes, the designated immunopharmacotherapeutic was infused slowly through intravenous catheters 3 min following a 50%-lethal cocaine injection, and the percentage of mice that developed symptoms of acute cocaine toxicity was calculated for each treatment group. The appearance and severity of ataxia, seizures, and lethality as an endpoint, were recorded across 3-min observation intervals for statistical analysis according to the methods established in the evaluation of murine mAb GNC92H2.¹⁶ It was previously reported that an LD₅₀ cocaine dose led to 28% lethality within the IgG-92H2-immunized group and 54% lethality across cocaine alone controls. Treatment with IgG-gzk lowered the rate of lethality to 20% among immunized mice (IgG-gzk, IgG-92H2: 180 mg/kg, see Fig. 2), and this anti-lethal effect was significant in comparison to the expected rate of mortality in the cocaine alone group ($\chi^2 = 4.9199$, $p < 0.05$).

Based on the equivalent therapeutic efficacy of both F(ab')₂-92H2 doses (132 mg/kg and 66 mg/kg), the mAb format dosing scheme was revised from that previously implemented by our laboratory during the examination of GNC92H2.¹⁶ Antidotal administration with a minimal F(ab')₂-gzk dose (66 mg/kg) did enable subjects' survival after their exposure to an LD₅₀ cocaine dose. Whereas neither IgG-92H2 nor IgG-gzk (180 mg/kg) significantly

altered the incidence of premorbid behaviors or seizures relative to their occurrence in unimmunized mice, F(ab')₂-gzk (66 mg/kg) treatment blocked the appearance of both symptomologies in a significant percentage of immunized mice (Fig. 2). 30% of F(ab')₂-gzk-immunized mice failed to develop premorbid symptoms apart from mild hyperactivity, while > 90% of unimmunized mice exhibited the initial behavioral effects of cocaine exposure. Likewise, F(ab')₂-gzk prevented seizure generation in 70% of mice while only 45% of cocaine-alone mice avoided this more severe sign of cocaine toxicity.

Effect of the human mAb GNCgzk IgG, F(ab')₂, (and Fab) formats on cocaine-induced premorbid behaviors and seizure generation

Fab-gzk was tested at two dosages: 60 mg/kg and 120 mg/kg, in order to compare its therapeutic ability to that of the most effective GNC92H2-based antidote, Fab-92H2 (120 mg/kg), as well as to extend our survey of minimally effective mAb doses. Whereas 65% of drug alone mice exhibited seizure activity as a result of acute cocaine toxicity, only 30% of immunized mice developed seizures in the Fab-gzk treatment groups (60 mg/kg, 120 mg/kg). This represented a level of protection that was statistically significant by χ^2 test of goodness-of-fit for the appearance of seizures in either Fab-gzk treatment group (seizure occurrence: yes/no, $\chi^2 = 5.27$, $p < 0.05$) relative to the expected distribution (i.e., the seizure activity within the cocaine alone group). This contrasted with the greater than 50% of mice in IgG-gzk, IgG-92H2, and F(ab')₂-92H2 (66 mg/kg and 132 mg/kg) treatment groups exhibiting seizures.¹⁶

The progression of cocaine overdose symptomologies was also assessed via scoring the severity of ataxic behaviors (0-to-5 scale) and seizures (0-to-3 scale) for each subject within successive 3-min time intervals and then calculating the mean score by treatment group within each interval. In addition, the highest seizure or ataxia score received by each subject during the 60-min observation period was noted, and the treatment group means for symptom severity were computed. Delivery of the LD₅₀ cocaine dose produced a maximum seizure score of 1.49 ± 0.18 (mean $\pm$ SEM) and a maximum ataxia score of 4.10 ± 0.21 (Table 1, Cocaine Alone group).

This methodology was applied to the analysis of group behavioral data upon antidotal immunization with the different GNCgzk formats. The ataxia and seizure scores for all treated mice across successive 3-min intervals were analyzed statistically by ANOVA and by the unpaired Student's *t* test for comparisons between two groups within specific intervals. A one-way ANOVA of ataxia and seizure scores confirmed that there was no significant difference in the onset of cocaine toxicity among different treatment groups during the 3-min interim between cocaine injection and passive vaccination [ataxia: $F(3,77) = 0.526$, $p > 0.5$; seizures: $F(3,77) = 1.111$, $p > 0.05$] (Figs. 3–4).

All GNCgzk-based antidotes significantly lowered the rate of lethality of an LD₅₀ cocaine dose. However, only F(ab')₂-gzk reduced the maximum severity of ataxia and seizures that was reached at the height of acute toxicity (Table 1), and significantly attenuated their development across multiple 3-min time intervals, which was concluded through the Student's *t* test comparison between cocaine-alone and F(ab')₂-gzk treatment group data (ataxia: maximum severity score, $p < 0.05$; 3–6 min, $p < 0.05$, 15–18 min, $p < 0.05$, 18–21 min, $p < 0.05$; seizures: mean severity, $p < 0.05$; 3–6 min, $p < 0.05$). The χ^2 goodness-of-fit test was implemented to assess whether the distribution of ataxia or seizure severity scores of mice within an immunized group differed from the expected severity score distribution (i.e., the cocaine-alone group severity score distribution). This analysis confirmed the significant effect of F(ab')₂-gzk treatment on all symptoms of severe toxicity (Table 2: ataxia, $\chi^2 = 25.99$, $p < 0.0001$; seizures, $\chi^2 = 7.60$, $p = 0.05$; lethality (alive/dead) $\chi^2 = 12.17$, $p < 0.0005$). By contrast, the same dose (66 mg/kg) of the murine mAb F(ab')₂-92H2 failed

to exert a statistically detectable effect on the severity of ataxic behaviors or seizures within the 60-min observation period (χ^2 goodness-of-fit test: $p > 0.05$) or within individual 3-min time intervals (Figs. 3–4; Student's t test, all intervals: $p > 0.05$).

Although the Fab fragment was the optimal format for GNC92H2-based overdose reversal, Fab-gzk was less successful in tempering the symptoms of acute toxicity. Fab-gzk treatment at the 60 mg/kg dose prevented lethality ($\chi^2 = 4.92$, $p < 0.05$), and both Fab-gzk doses attenuated the appearance of seizures in one time interval (Student's t test, Fab-gzk vs. cocaine-alone: 3–6 min, $p < 0.05$). The protracted ataxic behaviors, which appear after the initial severe symptoms of toxicity begin to taper off, were also moderated by Fab-gzk (60 mg/kg) infusion (Fig. 4, bottom graph; 12–15 min, $p < 0.05$; 15–18 min, $p = 0.05$). Likewise Fab-gzk (60 mg/kg) treatment altered the expected distribution of ataxia scores, which was assessed by χ^2 goodness-of-fit tests using cocaine-alone group data for the expected ataxia score distributions (Table 2; $\chi^2 = 19.27$, $p < 0.001$). From the scoring of maximum severity of seizures and ataxia across Fab-gzk treatment groups (Table I), only the 120 mg/kg dosage of Fab-gzk had a significant effect; the mean severity of ataxia was statistically distinguishable from the cocaine-alone group data by the Student's t test.

DISCUSSION

Prophylactic immunopharmacotherapy with the murine IgG-92H2 has been documented to lower the rate of lethality of an LD₅₀ cocaine dose in mice, however it failed to significantly alter the acute phenotype of premorbid behaviors or seizures.¹⁶ Replicating the conditions of this previous study, passive immunization with the human mAb GNCgzk IgG (IgG-gzk, 90 mg/kg, i.v.) 30 minutes before cocaine administration (93 mg/kg, i.p.) was found to prevent death in all subjects and to confer superior protection from acute toxicity in comparison to IgG-92H2 pre-treatment. Thus, it was hypothesized that GNCgzk-based antidotes would show a similar enhancement over their GNC92H2-based counterparts in halting the progression of overdose symptoms as measured in our mouse behavioral paradigm. In the current study, antidotal treatment with IgG-gzk (180 mg/kg, i.v.) mediated a greater reduction in the rate of lethality relative to IgG-92H2 (180 mg/kg, i.v.), which allowed us to speculate on the clinical potential of GNCgzk-based therapeutics. However, for their future translation to clinical use, GNCgzk-based antidotes could pose a separate health hazard at this exceedingly high dosage (e.g., 180 mg/kg) with respect to the maximum protein concentration and volume that is safe and well-tolerated in humans. With the substitution of smaller mAb fragments for the full IgG in the preclinical study of GNC92H2, the mAb treatment doses were adjusted to maintain a constant cocaine binding capacity across all antibody formats according to their size and number of cocaine binding sites: 2.4×10^{-6} mol cocaine per kg antibody.¹⁶ This research was then extended within the current study to the examination of GNC92H2 and GNCgzk at lower format doses (1.2×10^{-6} mol/kg drug-binding capacity), which permitted an assessment of the dose sensitivity of mAb-mediated protection for each immunopharmacotherapeutic manifold.

Previous studies on the application of anti-drug mAbs to addiction, drug-induced stereotyped behaviors, and/or drug toxicity have delineated the importance of maintaining a near equivalent molar ratio between drug dose and antibody binding capacity to maximize treatment efficacy, which translates to unattainably high mAb doses for cocaine overdose reversal.²⁴ This has since been refuted by the ability of immunopharmacotherapeutic agents against PCP and cocaine to partially counteract the symptoms of intoxication when a molar excess of drug was administered.^{3,5,25–27} Thus, we speculated that, for indications requiring treatment during periods of acute cardiovascular hyperactivity, minimizing the protein load and infusion volume of the immunopharmacotherapeutic would be critical to its therapeutic success. As a caveat, Fab-92H2 (120 mg/kg) was shown to effectively counteract an LD₅₀

cocaine dose, which was attributed to the ability of its favorable pharmacokinetic properties, such as its widespread distribution and rapid clearance, to compensate for any adverse side-effects stemming from its high dose.²⁸ Other mAb formats whose pharmacokinetic properties were less favorable showed limited ability to reverse acute cocaine toxicity. These included the IgG, which acquires its superfluous effector functions, larger size, and extended half-life from its Fc region, and the scFv of GNC92H2¹⁶, whose immediate clearance and relative instability are detrimental to this therapeutic application.

With our original discovery that murine mAb GNC92H2 could reverse the lethal effects of acute cocaine toxicity, we sought to probe the therapeutic potential of the human mAb GNCgzk in both its F(ab')₂ and Fab formats. In light of the aforementioned considerations, we were led to ask whether lower doses of an antibody with superior cocaine affinity (i.e., GNCgzk) could still produce significant reductions in the rate of lethality of an LD₅₀ cocaine dose despite the substantial molar excess of drug to antibody binding capacity (Table 1). In doing so, we would be examining the intriguing possibility that different drug use paradigms impose their own unique constraints on the minimally effective mAb dose and binding affinity. For example, under rodent models for drug self-administration, saturation of the binding capacity of elicited titers with the administered drug terminated the therapeutic effects of vaccination, a trend that has been highlighted in nicotine, heroin, and methamphetamine self-administration.^{29–32} Consequently, it was theorized that the drug binding capacity of an immunopharmacotherapeutic treatment would only need to match the molar drug dose for effectively combatting addictive behaviors. We contend that the reversal of acute drug toxicity is pharmacodynamically different because it entails vaccine-mediated sequestration of a higher drug dose over a shorter timeframe. Unlike in drug self-administration, which demands more thorough drug sequestration, immunopharmacotherapeutic treatments specific for methamphetamine, PCP, and cocaine toxicity are only required to significantly lower the net accumulation of drug in the brain to be therapeutically effective.^{16,33–35} Depending on the specific scenario of drug abuse, submolar concentrations of the anti-drug mAb are sufficient to prevent acute toxicity through attenuating the initial distribution of drug to the brain and reversing the drug concentration gradient to favor drug efflux from the brain.

To accompany data on the rate of lethality in mAb treatment groups, a critical comparison of the incidence and severity of cocaine-induced symptomologies, including ataxia and seizures, between all GNC92H2- and GNCgzk-treated mouse groups was undertaken. Fab-92H2 (120 mg/kg, i.v.) was previously found to confer the greatest benefit of all GNC92H2-based antidotes in tempering the severity of cocaine overdose phenotypes, with the maximum seizure and ataxia scores calculated to be 0.46 ± 0.28 and 2.09 ± 0.51 , respectively.¹⁶ Likewise, Fab-gzk (120 mg/kg, i.v.) was found to confer a significant reduction in the incidence and severity of seizures but not in the rate of lethality. When the dosages of the different GNC92H2 and GNCgzk treatments were reduced (2.4×10^{-6} mol/kg to 1.2×10^{-6} mol/kg mAb binding capacity for cocaine), the superiority of the GNCgzk F(ab')₂ format in overdose reversal was uncovered. Whereas a high-dose of F(ab')₂-92H2 and Fab-92H2 (2.4×10^{-6} mol/kg) significantly lowered the rate of lethality and the severity of ataxic symptoms, Fab-gzk and F(ab')₂-gzk treatments were capable of matching this level of protection at markedly lower doses (1.2×10^{-6} mol/kg). Furthermore, F(ab')₂-gzk guaranteed the survival of all cocaine-exposed subjects and significantly ameliorated the severity of seizures (Tables 1, 2: F(ab')₂ group data).

These disparate therapeutic effects of high-dose Fab-92H2 and F(ab')₂-92H2 versus low-dose Fab-gzk and F(ab')₂-gzk are readily interpreted by the counterbalance between the pharmacokinetic and pharmacodynamic properties of mAbs in shaping their antidotal value. Namely, the superior binding affinity of mAb GNCgzk may have compensated for the

cocaine binding capacity (1.2×10^{-6} mol/kg) of lower mAb doses. GNCgzk holds the highest affinity for cocaine ($K_d = 0.18$ nM) of *fully* human antibodies reported in the literature, with the principal candidates for cocaine immunopharmacotherapy including: murine mAbs GNC92H2 ($K_d = 2.0$ nM), 3P1A6 ($K_d = 0.22$ nM) and MM0240PA ($K_d = 11$ nM), and human/mouse chimeric mAb 2E2 ($K_d = 4$ nM).^{36,37} But, the therapeutic relevance of this trait was not fully realized until the evaluation of GNCgzk alongside a similar mAb candidate (i.e., GNC92H2), whose 10-fold lower affinity for cocaine allowed us to probe in a logical manner the influence of binding affinity on antibody-mediated cocaine sequestration *in vivo* (Fig. 2). Although we did not directly measure the effect of each mAb's binding kinetics on their respective therapeutic efficacies, it seems probable that the rate constants for mAb-cocaine binding as well as for mAb-cocaine complex decay may both impact the efficiency of mAb-mediated cocaine neutralization *in vivo* as it relates to overdose reversal. The kinetics of cocaine binding was measured for human mAb GNCgzk: $k_{on} = 4.3 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$, $k_{off} = 6.8 \times 10^{-3} \text{ s}^{-1}$. While mAb GNCgzk exhibited a moderate dissociation rate (k_{off}), which denotes the stability of the mAb-bound drug complex, its association rate (k_{on}) was impressively fast. The latter should enhance the ability of GNCgzk-based antidotes to treat time-sensitive indications such as acute overdose because it points to the ability of administered mAb to immediately recognize and bind any free cocaine that is encountered. In support of this hypothesis, GNCgzk-based treatments were optimal in terminating the progression of symptoms related to cocaine toxicity.

It follows that this depiction of overdose reversal by optimized formats of the human mAb GNCgzk disproves many of the supposed weaknesses of mAb-based treatments for drug abuse. All GNCgzk-based treatments prevented lethality from exposure to an LD₅₀ cocaine dose in a significant portion of immunized mice despite both the substantial molar excess of drug to antibody binding capacity and the acute nature of this behavioral paradigm, in which treatment is administered after the appearance of acute symptoms of toxicity. Of particular note, F(ab')₂-gzk tempered the severity of premorbid behaviors in addition to dampening seizure generation. This capability, which signifies the early blockade of severe excitotoxicity through mAb-mediated reductions of cocaine levels in the brain, could prove crucial to the long-term outcome of a patient's neurological health. In conclusion, from our investigations into numerous immunopharmacotherapeutic strategies for treating the various scenarios of cocaine abuse, F(ab')₂-gzk has distinguished itself as a passive vaccine holding the greatest clinical promise based on the synergistic balance between its intermediate pharmacokinetic profile relative to the IgG and scFv formats, the superior pharmacodynamic properties of mAb GNCgzk, and the physiological benefit of a minimally effective protein dose as an antidote for acute cocaine toxicity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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NONSTANDARD ABBREVIATIONS FOOTNOTE

mAb	monoclonal antibody
IgG	immunoglobulin G
F(ab')₂	two Fab fragments joined by disulfide bonds in the hinge region
Fab	fragment antigen-binding region of antibody
scFv	single-chain fragment, variable domain of antibody
V_H and V_L	antibody heavy- and light-chain variable domains
C_{H1} and C_Lκ	antibody heavy- and light-chain constant regions
Fc	fragment crystallizable region of antibody
mol. Eq	molar equivalent as it pertains to the antibody dose yielding a number of drug-binding sites that is equivalent to the administered drug dose (in molecules)
GNC-BSA or GNC-KLH	GNC hapten conjugated to bovine serum albumin carrier protein or keyhole limpet hemocyanin
i.v	intravenous
Cocaine Alone (group)	mouse group administered vehicle saline (i.v.) and LD ₅₀ dose cocaine (i.p.)
IgG-gzk	GNCgzk IgG-based passive vaccine
IgG-92H2	GNC92H2 IgG-based passive vaccine
F(AB')₂-gzk	GNCgzk F(ab') ₂ -based passive vaccine
F(AB')₂-92H2	GNC92H2 F(ab') ₂ -based passive vaccine
FAB-gzk	GNCgzk Fab-based passive vaccine
FAB-92H2	GNC92H2 Fab-based passive vaccine
scFv-92H2	GNC92H2 scFv-based passive vaccine
ELISA	enzyme-linked immunosorbent assay
LAL	limulus amoebocyte lysate assay
K_d	dissociation constant
t_{1/2}	elimination half-life
V_{dss}	volume of distribution at steady state
PCP	phencyclidine
CNS	central nervous system
BBB	blood-brain barrier
ANOVA	analysis of variance
Fisher's PLSD	Fisher's protected least significant difference
ns	not significant
NIDA	National Institute on Drug Abuse

References

1. Carrera MR, Ashley JA, Parsons LH, Wirsching P, Koob GF, Janda KD. Suppression of psychoactive effects of cocaine by active immunization. *Nature*. 1995; 378:727–730. [PubMed: 7501020]
2. Martell BA, Orson FM, Poling J, Mitchell E, Rossen RD, Gardner T, Kosten TR. Cocaine vaccine for the treatment of cocaine dependence: a randomized double-blind placebo-controlled efficacy trial. *Arch Gen Psych*. 2009; 66:1116–1123.
3. Kantak KM, Collins SL, Lipman EG, Bond J, Giovanoni K, Fox BS. Evaluation of anti-cocaine antibodies and a cocaine vaccine in a rat self-administration model. *Psychopharmacology*. 2000; 148:251–262. [PubMed: 10755738]
4. Tatsis N, Ertl HC. Adenoviruses as vaccine vectors. *Mol Ther*. 2004; 10:616–629. [PubMed: 15451446]
5. Carrera MRA, Trigo JM, Wirsching P, Roberts AJ, Janda KD. Evaluation of the anticocaine monoclonal antibody GNC92H2 as an immunotherapy for cocaine overdose. *Pharmacol Biochem Behav*. 2005; 81:709–714. [PubMed: 16005948]
6. Carrera MR, Ashley JA, Wirsching P, Koob GF, Janda KD. A second-generation vaccine protects against the psychoactive effects of cocaine. *Proc Natl Acad Sci USA*. 2001; 98:1988–92. [PubMed: 11172063]
7. Carrera MR, Ashley JA, Zhou B, Wirsching P, Koob GF, Janda KD. Cocaine vaccines: antibody protection against relapse in a rat model. *Proc Natl Acad Sci USA*. 2000; 97:6202–6. [PubMed: 10823960]
8. Baird TJ, Deng SX, Landry DW, Winger G, Woods JH. Natural and artificial enzymes against cocaine. I. Monoclonal antibody 15A10 and the reinforcing effects of cocaine in rats. *J Pharmacol Exp Ther*. 2000; 295:1127–1134. [PubMed: 11082449]
9. Haney M, Gunderson EW, Jiang H, Collins ED, Foltin RW. Cocaine-specific antibodies blunt the subjective effects of smoked cocaine in humans. *Biol Psychiatry*. 2010; 67:59–65. [PubMed: 19846066]
10. Kosten TR, Rosen M, Bond J, Settles M, Roberts JSC, Shields J, Jack L, Fox B. Human therapeutic cocaine vaccine: safety and immunogenicity. *Vaccine*. 2002; 20:1196–1204. [PubMed: 11803082]
11. Martell BA, Mitchell E, Poling J, Gonsai K, Kosten TR. Vaccine Pharmacotherapy for the Treatment of Cocaine Dependence. *Biological Psychiatry*. 2005; 58:158–164. [PubMed: 16038686]
12. Cerny EH, Cerny T. Anti-nicotine abuse vaccines in the pipeline: an update. *Expert Opin Investig Drugs*. 2008; 17:691–696.
13. McKenzie SJ, Halsey JF. Cholera toxin B subunit as a carrier protein to stimulate a mucosal immune response. *J Immunol*. 1984; 133:1818–1824. [PubMed: 6470484]
14. Kinsey BM, Jackson DC, Orson FM. Anti-drug vaccines to treat substance abuse. *Immunol Cell Biol*. 2009; 87:309–314. [PubMed: 19333250]
15. Carrera MRA, Meijler MM, Janda KD. Cocaine pharmacology and current pharmacotherapies for its abuse. *Bioorg Med Chem*. 2004; 12:5019–5030. [PubMed: 15351386]
16. Treweek JB, Roberts AJ, Janda KD. Immunopharmacotherapeutic manifolds and modulation of cocaine overdose. *Pharmacol Biochem Behav*. 2011; 98:474–484. [PubMed: 21356233]
17. Mendez MJ, Green LL, Corvalan JRF, Jia XC, Yang XD, Gallo ML, Louie DM, Lee DV, Erickson KL, Luna J, Roy CMN, Abderrahim H, Kirschenbaum F, Noguchi M, Smith DH, Fukushima A, Hales JF, Finer MH, Geoffrey Davis C, Zsebo KM, Jakobovits A. Functional transplant of megabase human immunoglobulin loci recapitulates human antibody response in mice. *Nat Genet*. 1997; 15:146. [PubMed: 9020839]
18. Green LL. Antibody engineering via genetic engineering of the mouse: XenoMouse strains are a vehicle for the facile generation of therapeutic human monoclonal antibodies. *J Immunol Methods*. 1999; 231:11–23. [PubMed: 10648924]
19. Larsen NA, Zhou B, Heine A, Wirsching P, Janda KD, Wilson IA. Crystal structure of a cocaine-binding antibody. *J Mol Biol*. 2001; 311:9–15. [PubMed: 11469854]

20. Steiniger SC, Altobelli LJr, Zhou B, Janda KD. Selection of human antibodies against cell surface-associated oligomeric anthrax protective antigen. *Mol Immunol.* 2007; 44:2749–2755. [PubMed: 17210180]
21. Ritz MC, George FR. Cocaine-induced seizures and lethality appear to be associated with distinct central nervous system binding sites. *J Pharmacol Exp Ther.* 1993; 264:1333–1343. [PubMed: 8450469]
22. Hearn WL, Rose S, Wagner J, Ciarleglio A, Mash DC. Cocaethylene is more potent than cocaine in mediating lethality. *Pharmacol Biochem Behav.* 1991; 39:531–533. [PubMed: 1946594]
23. Sturgeon RD, Fessler RG, Meltzer HY. Behavioral rating scales for assessing phencyclidine-induced locomotor activity, stereotyped behavior and ataxia in rats. *Eur J Pharmacol.* 1979; 59:169–179. [PubMed: 575093]
24. Treweek JB, Roberts AJ, Janda KD. Superadditive effects of ethanol and flunitrazepam: implications of using immunopharmacotherapy as a therapeutic. *Mol Pharm.* 2010; 7:2056–2068. [PubMed: 20849117]
25. Proksch JW, Gentry WB, Owens SM. Anti-phencyclidine monoclonal antibodies provide long-term reductions in brain phencyclidine concentrations during chronic phencyclidine administration in rats. *J Pharmacol Exp Ther.* 2000; 292:831–837. [PubMed: 10688594]
26. Laurenzana EM, Gunnell MG, Gentry WB, Owens SM. Treatment of adverse effects of excessive phencyclidine exposure in rats with a minimal dose of monoclonal antibody. *J Pharmacol Exp Ther.* 2003; 306:1092–1098. [PubMed: 12829731]
27. Pitas G, Laurenzana EM, Williams DK, Owens SM, Gentry WB. Anti-phencyclidine monoclonal antibody binding capacity is not the only determinant of effectiveness, disproving the concept that antibody capacity is easily surmounted. *Drug Metab Dispos.* 2006; 34:906–912. [PubMed: 16507651]
28. Hawthorne, N. *The Scarlett Letter*. Ticknor, Reed & Fields; Boston: 1850.
29. Pentel PR, Malin DH, Ennifar S, Hieda Y, Keyler DE, Lake JR, Milstein JR, Basham LE, Coy RT, Moon JW, Naso R, Fattom A. A nicotine conjugate vaccine reduces nicotine distribution to brain and attenuates its behavioral and cardiovascular effects in rats. *Pharmacol Biochem Behav.* 2000; 65:191–198. [PubMed: 10638653]
30. Bonese KF, Wainer BH, Fitch FW, Rothberg RM, Schuster CR. Changes in heroin self-administration by a rhesus monkey after morphine immunisation. *Nature.* 1974; 252:708–710. [PubMed: 4474602]
31. Laurenzana EM, Byrnes-Blake KA, Milesi-Hallé A, Gentry WB, Williams DK, Owens SM. Use of anti-(+)-methamphetamine monoclonal antibody to significantly alter (+)-methamphetamine and (+)-amphetamine disposition in rats. *Drug Metab Dispos.* 2003; 31:1320–1326. [PubMed: 14570763]
32. Peterson EC, Laurenzana EM, Atchley WT, Hendrickson HP, Owens SM. Development and preclinical testing of a high-affinity single-chain antibody against (+)-methamphetamine. *J Pharmacol Exp Ther.* 2008; 325:124–133. [PubMed: 18192498]
33. Valentine JL, Mayersohn M, Wessinger WD, Arnold LW, Owens SM. Antiphencyclidine monoclonal Fab fragments reverse phencyclidine-induced behavioral effects and ataxia in rats. *J Pharmacol Exp Ther.* 1996; 278:709–716. [PubMed: 8768722]
34. Norman AB, Tabet MR, Norman MK, Buesing WR, Pesce AJ, Ball WJ. A chimeric human/murine anticocaine monoclonal antibody inhibits the distribution of cocaine to the brain in mice. *J Pharmacol Exp Ther.* 2007; 320:145–153. [PubMed: 17023573]
35. Fox BS, Kantak KM, Edwards MA, Black KM, Bollinger BK, Botka AJ, French TL, Thompson TL, Schad VC, Greenstein JL, Geftter ML, Exley MA, Swain PA, Briner TJ. Efficacy of a therapeutic cocaine vaccine in rodent models. *Nat Med.* 1996; 2:1129–1132. [PubMed: 8837612]
36. Paula S, Tabet MR, Keenan SM, Welsh WJ, Ball WJJ. Three-dimensional structure-activity relationship modeling of cocaine binding to two monoclonal antibodies by comparative molecular field analysis. *J Mol Biol.* 2003; 325:515–530. [PubMed: 12498800]
37. Paula S, Tabet MR, Farr CD, Norman AB, Ball WJJ. Three-dimensional quantitative structure-activity relationship modeling of cocaine binding by a novel human monoclonal antibody. *J Med Chem.* 2004; 47:133–142. [PubMed: 14695827]

Figure 1.

Therapeutic benefit of prophylactic immunization against cocaine. Treatments (Cocaine Alone: 10 mL/kg saline, IgG-92H2: 90 mg/kg, IgG-gzk: 90 mg/kg; i.v.) were administered 30 min before cocaine injection (93 mg/kg, i.p.), and the occurrence of cocaine-induced symptomologies was recorded during the 60-min observation period. Data are expressed as a percentage of mice within each treatment group that exhibited premorbid behaviors, seizures or lethality after receipt of the LD₅₀ cocaine dose (†, no lethality).

Figure 2.

Comparison between human mAb GNCgzk-mediated and murine mAb GNC92H2-mediated prevention of cocaine-induced toxicity. Treatments (Cocaine Alone: 10 mL/kg saline, IgG: 180 mg/kg, F(ab')₂: 66 mg/kg; i.v.) were administered 3 minutes after cocaine injection (93 mg/kg, i.p.). Data are expressed as a percentage of mice in each treatment group that experiences seizures or lethality from an LD₅₀ cocaine dose (†, no lethality).

Figure 3.

Time-dependent severity of cocaine-induced seizures in cocaine alone and immunized groups. An LD₅₀ dose cocaine was i.p. injected in mice at the start (time = 0 min) of behavioral monitoring, and 3 minutes into the observation period, the designated mAb treatment (top graph: IgG 180 mg/kg; middle graph: F(ab')₂ 66 mg/kg; bottom graph: Fab 120 mg/kg, Fab-gzk 60 mg/kg) or vehicle (Cocaine Alone: sterile 0.9% saline) was infused through the indwelling jugular vein catheter. Seizure activity (0–3 scale) was scored for each mouse across successive 3-min intervals and values for each time interval represent group mean scores ± S.E.M. for cocaine alone (dashed line) and immunized (solid line) groups. **p* < 0.05, for the Student's *t* test comparison of cocaine alone versus immunized groups within designated time intervals.

Figure 4.

Effect of GNCgzk-based versus GNC92H2-based immunopharmacotherapeutics on cocaine-induced ataxia. Subjects were administered an LD₅₀ cocaine dose by i.p. injection at time = 0 min, upon which each subject was transferred to a locomotor activity cage for behavioral monitoring. The assigned GNC92H2 (triangle) or GNCgzk (square) format treatment (top graph: IgG, 180 mg/kg; middle graph: F(ab')₂, 66 mg/kg; bottom graph: Fab, 60 and 120 mg/kg) was infused through the jugular vein catheter at time = 3–4 min. Subjects in the cocaine alone group (diamond) were administered vehicle (sterile 0.9% saline, i.v.). Ataxia (0–5 scale) was scored for each subject within successive 3-min intervals for the 36-min observation period, and group scores were averaged within each 3-min interval (mean ± S.E.M.) for comparison of ataxic symptoms between the cocaine alone (dashed line) and immunized groups (solid line). *, † *p* < 0.05, for the Student's *t* test comparison of cocaine alone versus immunized groups within designated time intervals.

Summary of ataxia and seizure severity scores in cocaine control and immunized mice upon injection of an LD₅₀ cocaine dose. Data represents group mean $\pm$ S.E.M. of the maximum ataxia (0–5) or seizure (0–3) score attained by each subject within the 60-min observation period.

Table 1

	Cocaine Alone	IgG	F(ab') ₂	Fab
	(93 mg/kg)	(180 mg/kg) ^a	(66 mg/kg) ^b	(120 mg/kg) ^a (60 mg/kg) ^b
Ataxia	4.10 $\pm$ 0.21	3.70 $\pm$.56	2.70 $\pm$ 0.78 [*]	4.60 $\pm$ 0.22 3.60 $\pm$ 0.54
Seizure	1.49 $\pm$ 0.18	1.60 $\pm$ 0.45	0.50 $\pm$ 0.31 [*]	0.60 $\pm$ 0.34 [*] 0.80 $\pm$ 0.42

^{*} $p < 0.05$ for the Student's t test comparison of cocaine alone versus immunized group scores.

^a Antibody binding capacity for cocaine: 2.4×10^{-6} mol/kg

^b Antibody binding capacity for cocaine: 1.2×10^{-6} mol/kg

Table 2

Statistical analysis of changes to cocaine-induced symptomology by 3-min post-treatment with mAb GNCgzk IgG^a, F(ab')₂^b, and Fab (60^b, 120^a mg/kg).

Symptomology	Group	χ^2 Test of Goodness-of-Fit
Death (alive or dead)	IgG	$\chi^2 = 4.92, p < 0.05$
	F(ab') ₂	$\chi^2 = 12.17, p < 0.0005$
	Fab ^a	<i>ns</i> , $p < 0.06$
	Fab ^b	$\chi^2 = 4.92, p < 0.05$
Seizure Score (0,1,2,3)	IgG	<i>ns</i> ^c
	F(ab') ₂	$\chi^2 = 7.60, p < 0.05$
	Fab ^a	<i>ns</i>
	Fab ^b	<i>ns</i>
Ataxia Score (0,1,2,3,4,5)	IgG	<i>ns</i>
	F(ab') ₂	$\chi^2 = 25.99, p < 0.0001$
	Fab ^a	<i>ns</i>
	Fab ^b	$\chi^2 = 19.27, p < 0.001$

^a Antibody binding capacity for cocaine: 2.4×10^{-6} mol/kg

^b Antibody binding capacity for cocaine: 1.2×10^{-6} mol/kg

^c *ns*: not significant