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The effects of a repeated dose of a recombinant humanized anti-cocaine monoclonal antibody on cocaine self-administration in rats

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Abstract

Background—Immunotherapy has shown potential as a treatment for cocaine abuse. The humanized recombinant anti-cocaine monoclonal antibody (mAb) with the preclinical designation h2E2 has been shown to decrease cocaine concentrations in the brain in rats, but its effects on cocaine self-administration behavior have never been tested.

Methods—The amount of cocaine needed to reinstate self-administration behavior (priming threshold) was calculated and the inter-injection intervals at unit doses of 0.3 $\mu\text{mol/kg}$ and 3 $\mu\text{mol/kg}$ during maintained self-administration were measured over a five-week baseline period. Rats trained to self-administer cocaine were infused with two doses of h2E2 (120 mg/kg i.v.) 35 days apart. Priming threshold and inter-injection intervals were measured for 35 days after both injections.

Results—After both injections of h2E2, priming thresholds were significantly increased (3-fold) compared to expected baseline and then gradually declined over 35 days. A significant decrease (15–33%) in inter-injection intervals during maintained self-administration was also observed following both h2E2 at the lower dose, and after the first injection at the higher dose. No significant decreases in body weight were observed after either injection, indicating a lack of toxicity following a second injection.

Conclusions—These data predict that the safety and efficacy of h2E2 will be maintained after multiple treatments of this potential immunotherapy for cocaine abuse.

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Conflict of Interest

Dr. Norman is named as a co-inventor on a patent application for the matter and use of the h2E2 humanized anti-cocaine monoclonal antibody.

Contributors

All authors have read and approved the final manuscript.

Participated in research design: Wetzel, Tsibulsky, Norman

Conducted experiments: Wetzel, Tsibulsky

Performed data analysis: Wetzel, Tsibulsky

Wrote or contributed to the writing of the manuscript: Wetzel, Tsibulsky, Norman

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Keywords

immunotherapy; priming threshold; reinstatement; addiction; translational research

1. INTRODUCTION

Cocaine reliably induces the reinstatement of self-administration behavior in rats and has been suggested to partially model relapse in cocaine addicts (Stewart, 1983; Norman et al., 1999). The minimal amount of cocaine needed to reinstate self-administration behavior was termed the priming threshold (Norman et al., 1999, 2002). Therefore, the cocaine concentration at the site of action is a critical determinant of reinstatement of self-administration behavior (Norman et al., 1999). An increase in the cocaine priming threshold is predicted to decrease the probability of a cocaine-induced relapse. One method for decreasing the amount of cocaine at its site of action (dopamine transporters in the brain) is the use of anti-cocaine antibodies, which bind to cocaine and prevent its distribution into the brain. This bound cocaine is assumed to be pharmacodynamically inert. Cocaine vaccines that induce the production of anti-cocaine antibodies have been shown to decrease cocaine use in clinical trials, but only in patients that produce high antibody titers. The responses to these vaccines are highly variable, and since their effectiveness is directly related to antibody titers, the clinical response to these medications is not uniform (Martell et al., 2009; Kosten et al., 2014). Similarly, an anti-nicotine vaccine has shown some effectiveness in clinical trials (Keyler et al., 2005), but only if high anti-nicotine titers are elicited.

An alternative to active immunization using vaccines is the use of passive immunization with anti-cocaine antibodies. Since the main predictor of success of cocaine vaccines is the amount of antibody raised, injection of known doses of a high-affinity anti-cocaine monoclonal antibody (mAb) should provide a more consistent and reliable therapeutic effect. An anti-methamphetamine mAb has shown effectiveness in animal models of methamphetamine abuse (Laurenzana et al., 2003) and has advanced into clinical trials (Stevens et al., 2014). A chimeric anti-cocaine mAb 2E2 has been effective in decreasing cocaine concentrations in the brain in mice (Norman et al., 2007) and has raised the cocaine priming threshold in rats (Norman et al., 2009). This was interpreted as a decrease in the probability of a dose of cocaine reinstating self-administration behavior. The recombinant humanized anti-cocaine mAb 2E2 was reengineered, and the humanized h2E2 is now a lead candidate for development as a passive immunotherapy for cocaine abuse. It shares greater than 95% sequence homology with the human IgG₁, has high affinity ($K_d=4.1-16$ nM) and selectivity for cocaine, and can now be produced in gram quantities using a stably transfected mammalian cell line (Norman et al., 2014; Kirley and Norman, 2015). Although a single infusion of this mAb has been shown to prevent cocaine entry into the brain in rats (Norman et al., 2014), the effects of h2E2 on cocaine self-administration behavior have not previously been investigated. Additionally, long-term treatments for relapse prevention will require multiple doses, but the effects of a repeat injection of h2E2 nor 2E2 have been studied. Therefore, the aim of this study was to determine the effects of h2E2 on cocaine induced reinstatement of self-administration and maintained self-administration in rats and to investigate whether a repeat dose remains effective.

2. MATERIALS AND METHODS

2.1 Animals

17 male Sprague-Dawley rats between 200 g and 500 g during the course of this study were purchased from Harlan Laboratories (Indianapolis, IN). Rats were housed individually on a 14/10-hour light/dark cycle with unrestricted access to food and water. All studies were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (Institute for Laboratory Animal Research, 2011) and under a protocol approved by the Institutional Animal Care and Use Committee at the University of Cincinnati.

2.2 Self-administration training

Rats were implanted with indwelling catheters into the right jugular vein under isoflurane anesthesia. If recatheterization was required, catheters were placed in the left jugular and femoral veins as needed throughout the study. Buprenorphine (0.03 mg/rat s.c) was administered post-surgery for pain control and gentamycin (25 mg/rat s.c) for three days was used to prevent infection following surgery. Detailed protocols for cocaine self-administration training can be found in (Tsibulsky and Norman, 2005). In brief, beginning at least 5 days after surgery, rats were trained to self-administer cocaine HCl. Rats were weighed immediately prior to each self-administration session. Animals were placed in isolated chambers containing an active and an inactive lever. During training, a unit dose of 3 μ mol/kg (1 mg/kg) of cocaine HCl was delivered on a fixed-ratio 1 (FR1) schedule with at least a 5 second timeout period. A cue light was illuminated for the duration of the timeout. Rats had access to cocaine for 3 hours a day, five days a week. Training was considered complete when inter-injection intervals did not systematically deviate (defined as less than $\pm 10\%$ variance (standard deviation) in the mean inter-injection interval of a standard unit dose of cocaine (3 μ mol/kg)) from day to day for three consecutive sessions.

2.3 Priming threshold

Sessions began between 8:00 and 10:00 AM, 6 days a week. Priming threshold, defined as the minimal level of cocaine that reinstates self-administration behavior, was estimated using programmed escalating doses of cocaine to raise the concentration in the rat until self-administration was reinstated. A procedure modified from Norman et al (1999, 2009) was used. In our previous studies, the concentration of cocaine during the priming phase of the session was increased linearly. In this study, the concentration was increased according to a sigmoid logistic function with the maximum level set at 20 μ mol/kg to prevent overdose if the rat never met the criterion for reinstatement.

First, rats were placed in the chamber and a cue light associated with cocaine injection was illuminated after every active lever press and at variable intervals of 100 – 600 s until no lever presses occurred for at least 30 min. This was done to eliminate the interference of cue-induced lever pressing on the measurement of priming threshold. Once cue-induced lever-pressing was extinguished, programmed non-contingent injections of cocaine were given every two minutes at escalating doses in order to raise the concentration of cocaine. When the rat pressed the active lever 5 times with each interval shorter than 2 min, it was defined

that self-administration had been reinstated and programmed injections ceased. Priming threshold was determined by averaging the calculated peak cocaine levels after the second to last and last priming injections.

2.4 Maintained self-administration and extinction

After self-administration was reinstated, the program allowed the rat 75 injections of 0.3 $\mu\text{mol/kg}$ and then 15 injections of 3 $\mu\text{mol/kg}$. When these injections were complete, the syringe pump was inactivated and lever pressing was recorded but did not result in an injection. Animals were left in their chambers until 30 minutes had passed since their last lever press, at which time session was ended. Animals were then removed from the chamber and returned to their home cages until the next session.

2.5 Calculations of cocaine concentration in the body

Complete protocols for the calculation of cocaine in the rats' bodies can be found in Tsibulsky and Norman (2005). Briefly, the drug level in the body was calculated every second using a one-compartment pharmacokinetic model with an assumption of a 500 s elimination half-life of cocaine.

2.6 Quantification of cocaine and benzoylecgonine following maintained self-administration

A different set of rats trained to self-administer cocaine was given access to cocaine (fixed ratio FR1) with a unit dose of 3 $\mu\text{mol/kg}$ for 4–5 hours. At the end of a session, a small incision was made at the tip of the tail and 10–50 μL of blood was collected. Cocaine and BE were extracted using solid phase extraction and quantified using GC/MS as previously reported in Norman et al (2007).

2.7 h2E2 infusions

The mAb h2E2, concentrated to 17 mg/mL in phosphate buffered saline (pH 7), was infused using the same apparatus used for the self-administration studies. Antibody (120 mg/kg, i.v.) was infused at a rate of 162 $\mu\text{L/min}$, where the duration of infusion was adjusted for the rats' body weight. Infusions lasted approximately 20 min.

2.8 Data analysis and statistics

Due to multiple catheter failures, six out of twelve rats were eliminated from the experiment before the first h2E2 injection and one rat was eliminated before the second injection of antibody. Every daily session generated four measurements: the body weight, the priming threshold, and the mean inter-injection interval at two cocaine unit doses (0.3 and 3.0 $\mu\text{mol/kg}$). Baseline priming threshold and inter-injection interval values within sessions were typically log-normally distributed. Therefore, all statistical analyses of these two parameters were performed using logarithmic values. Baseline trends were determined for at least 5 weeks prior the first h2E2 injection. Regression analyses were performed using SigmaPlot (Systat Software, Inc., CA) and expected values for the days after injections were computed. All data were analyzed using paired t-tests comparing expected values generated by linear regression through all baseline data points for each individual rat to the actual values after

injection of h2E2. To correct for multiple comparisons, a false-discovery rate procedure was applied to all behavioral data according to methods found in Curran-Everett (2000). Cocaine concentrations were compared to BE concentrations using a paired t-test.

2.9 Materials

(–) Cocaine HCl was provided by the Research Triangle Institute (Chapel Hill, NC) under the National Institute on Drug Abuse Drug Supply Program. Recombinant h2E2 was produced from stably transfected CHO-cell lines by Catalent PharmaSolutions (Madison, WI) using their proprietary GPEX technology (Bleck, 2012). Buprenorphine, gentamycin, isoflurane and heparin were purchased from Henry Schein Animal Health (Dublin, OH).

3. RESULTS

3.1 The effects of h2E2 on priming thresholds

Priming thresholds followed linear trends of either an increase or decrease depending on the rat over the 5 week baseline period. The average slope of the regression line for all rats was negative and not significant ($y = 3.1652 - 0.0004 \cdot x$ and $y = 3.1661 - 0.0005 \cdot x$ on logarithmic scale, for $n = 6$ before and $n = 5$ after the second injection, respectively) (Fig. 1). Significant differences in priming threshold were observed after both injections. Both injections of h2E2 were equally effective increasing the priming threshold by 3.0 and 3.6-fold, respectively (Fig. 1). Over time, priming threshold values returned exponentially to the baseline.

3.2 The effects of h2E2 on maintained self-administration behavior

Inter-injection intervals during maintained self-administration at two different unit doses of cocaine, 0.3 and 3.0 $\mu\text{mol/kg}$, were measured. Geometric mean intervals followed linear trends of either an increase or decrease in different rats over the 5 week baseline period. (on a logarithmic scale, $y = 1.5322 + .0001 \cdot x$ and $y = 1.4858 + 0.0004 \cdot x$ at the 0.3 $\mu\text{mol/kg}$ dose before and after the second injection, respectively; $y = 2.4960 + .0002 \cdot x$ and $y = 2.4713 + 0.0002 \cdot x$ at the 3 $\mu\text{mol/kg}$ dose before and after the second injection, respectively). The average slope of the regression line for all rats was negative and insignificant (Fig. 2). Significant differences in inter-injection intervals were observed after both injections.

One day after the first h2E2 infusion, intervals had decreased by 33% and 25% for the 0.3 and 3.0 $\mu\text{mol/kg}$ doses, respectively, compared to the last day of baseline. Inter-injection intervals at both unit doses of cocaine were significantly decreased (Fig. 2). One day after the second h2E2 injection, intervals had decreased by 24% and 15% for the low and high doses, respectively, compared to the previous day. However, after the second injection, significant differences were seen only at the low dose (Fig. 2).

3.3 h2E2 has no effect on rat body weight

Animal's body weight increased over the duration of the experiment from 225 g to 500 g. The general trend can be approximated by a logarithmic function $y = a \cdot \ln(x - x_0)$ with normal fluctuation from the mean in the range of $\pm 0.5\%$. Surgical re-implantation of the intravenous catheter resulted in a statistically insignificant decrease in body weight within a

– 0.6% range. Even smaller insignificant decreases in body weight were observed following either injection of h2E2.

3.4 Cocaine and BE concentrations following self-administration

BE concentrations were significantly higher than cocaine concentrations at the end of a 4 – 5 hour self-administration session ($p = 0.02$). Mean $\pm$ SEM cocaine concentrations were 4.16 ± 0.41 $\mu\text{mol/mL}$ compared with BE concentrations of 7.90 ± 1.1 $\mu\text{mol/mL}$ ($n=5$). BE concentrations by the end of a cocaine self-administration session were, therefore, on average, 1.9 ± 0.26 -fold higher than cocaine concentrations.

4. DISCUSSION

The effects of immunotherapy on self-administration behavior vary between and within studies. For example, depending on both the antibody dose and cocaine dose, contradictory results have been achieved. In the following situations, decreases in the rate of self-administration were observed: 1) high doses of a catalytic anti-cocaine mAb using low cocaine unit doses (Baird et al., 2000); 2) an anti-cocaine mAb at all cocaine unit doses (Fox et al., 1996; Fox, 1997; Kantak et al., 2000); and 3) relatively high doses of the mAb GNC92H2 (Carrera et al., 2000). However, all of the following caused increases in the rate of self-administration 1) the same catalytic antibody at low antibody doses and high cocaine unit doses, 2) the mAb GNC92H2, and 3) a different high affinity mAb. A detailed discussion of the effects of the various experimental protocols on the effects of anti-cocaine antibodies on cocaine self-administration behavior is reviewed elsewhere (Wetzel et al., 2016). The methods used in the present study were designed to avoid potential confusion by clearly delineating between the reinstatement/loading phase, the maintenance phase, and the extinction phase of the session, which was not done in other studies. The procedures used here was not meant to model the exact conditions experienced by human addicts, instead it was designed to measure the pharmacodynamic potency of h2E2 over time. The procedure used to measure the cocaine priming threshold represents a titration, where the cocaine concentration in the rat is gradually increased until the priming-threshold is reached. While this does not mirror the human situation, it will provide accurate measures of the increase in the amount of cocaine needed to reinstate self-administration behavior, a value which likely is relevant in a relapse situation. However, the translational predictive validity of reinstatement models in general has been questioned (Katz and Higgins, 2003; Haney and Spealman, 2008; Schwandt et al., 2016). Only when clinical trials are eventually conducted will the translational validity of our assay system be confirmed or refuted.

In this study, cocaine priming threshold was increased by 3-fold. This is consistent with the demonstrated ability of h2E2 to substantially decrease the rat brain cocaine concentration after a single i.v. injection of cocaine (Norman et al., 2014). Since the pharmacodynamic mechanisms underlying self-administration behavior presumably remain unchanged by h2E2, more cocaine injected into the periphery is required to achieve high enough brain concentrations to reinstate self-administration. Consistent with h2E2's long elimination half-life in rats (7.8 days) (Norman et al., 2014), this effect was long lasting and gradually returned to baseline. An equally robust effect on priming threshold was observed after the

second injection of h2E2 in the present study. Interestingly, the observed change was slightly larger than the magnitude of change that would be expected based on the stoichiometry of cocaine to h2E2. When concentrations of cocaine and h2E2 are equal then the fractional occupancy of anti-cocaine binding sites is theoretically close to 94%. The 120 mg/kg dose of h2E2 is capable of binding up to 1.6 $\mu\text{mol/kg}$ of cocaine. The baseline level of the priming threshold in this study was approximately 1.2 $\mu\text{mol/kg}$. Therefore, the free concentration of cocaine would start to increase after injection of 1.6 $\mu\text{mol/kg}$ of cocaine and the rats will not prime until free cocaine concentration reach 1.2 $\mu\text{mol/kg}$. The total amount of cocaine required to reinstate the cocaine self-administration would be 2.3-fold higher in the presence of mAb. Interestingly, the effect observed in this study was slightly larger than theoretically predicted. This is consistent with what was observed using an anti-phencyclidine (PCP) mAb, where the mAb was capable of sequestering large amounts PCP in the blood, despite that on a mole basis it should not have been able to bind all the PCP present in the body (Proksch et al., 2000).

A significant decrease in inter-injection intervals during the maintenance phase of self-administration was measured at both cocaine doses after the first h2E2 injection, and at the lower unit dose following the first injection. However, a statistically significant change on inter-injection intervals at the higher unit dose of cocaine was not detectable following the second h2E2 injection. Consequently, it is possible that the effect of the second h2E2 dose is slightly reduced. This effect is similar to what was seen using h2E2's chimeric predecessor 2E2, where a larger effect on inter-injection intervals was also observed at the lower dose. It was hypothesized that this is because the percent of total cocaine that is bound to the antibody is larger at the lower dose (Norman et al., 2009). Again, if it is assumed that the pharmacodynamic mechanisms underlying this behavior were unchanged, it is not surprising that there is a modest decrease in inter-injection intervals. In clinical trials, an increase in cocaine intake (measured by cocaine and BE in the urine) would be interpreted as a negative outcome, or failure of the treatment (Martell et al., 2005). Therefore, this decrease in inter-injection intervals is not a clinically desirable effect, but the magnitude of this change is much smaller (15–33%) than the effect on priming threshold (300%), and it is predicted that brain concentrations would not be raised. It should also be noted that in this model rats were on an FR-1 schedule during this phase of the session, which is not representative of the human situation, so it is unclear whether or not this acceleration in cocaine consumption would be expected in humans. However, this could still represent a limitation of immunotherapies for cocaine abuse.

The much smaller effect of h2E2 on maintained self-administration as compared to priming threshold can also, in part, be explained by the large difference in plasma BE concentrations compared to cocaine concentrations that occur during maintained self-administration. In rats, the majority of cocaine is converted to BE. Since BE has a longer half-life than cocaine (Misra et al., 1975), it would be expected that the pseudo-steady state concentrations achieved during maintained self-administration would be higher for BE than cocaine after the rats have already primed. In fact, plasma concentrations of BE were 1.8-fold higher than cocaine. This is similar to what was reported under a more limiting (FR5) schedule (Bystrowska et al., 2012). Due to h2E2's lower but still moderate affinity (6.8-fold lower than cocaine) for BE (Norman et al., 2014), this accumulation of BE may neutralize a

portion of the h2E2 by competing with cocaine, further increasing the ratio between free cocaine and free h2E2. This neutralization may contribute to preventing an adverse increase in the rate of cocaine consumption. h2E2 is likely to be fully effective during the reinstatement phase of self-administration where BE levels are likely to be negligible. However, after the maintenance phase begins, some of the h2E2 could be bound by the increasing concentrations of BE produced by the metabolism of multiple doses of cocaine. This would decrease the binding capacity for cocaine and decrease the effect on the acceleration of self-administration. This is essentially a self-correcting system, when once the antibody has been surmounted then binding to BE would prevent a large increase in the rate of cocaine consumption. This implies that the concept of creating antibodies highly selective for the active drug may not be optimal, and an antibody with moderate affinity for the inactive metabolites may prevent the possible increase in the rate of cocaine self-administration. This hypothesis could be tested by using the more selective anti-cocaine antibody GNCgzk (Eubanks et al., 2014).

Weight loss is a commonly used indicator of toxicity in rodents, and body weight measurements are typically included in *Investigational New Drug Application to the Food and Drug Administration*. In a previous study in rats given a single bolus dose of cocaine, h2E2 increased plasma cocaine concentrations 17-fold (Norman et al., 2014). Though this cocaine is expected to be pharmacodynamically inert it is possible that combined with the increased cocaine consumption seen in this study, peripheral toxicity of cocaine could be induced. However, there is no evidence for this toxicity using body weight as a measure following either injection. This indicates that the combination of cocaine and h2E2 may not cause any detectable adverse health effects. This may indicate a favorable safety profile for this drug in clinical use even following multiple doses.

It is well known that large proteins have the capacity to induce immune responses, and this can occur in response to therapeutic antibodies in humans (Hwang and Foote, 2005). In humans, production of these antibodies may or may not interfere with the efficacy of the biologic drug (Schellekens, 2002). Therefore, if h2E2 induces an immune response, its pharmacodynamic and/or pharmacokinetic potencies could be decreased following a repeated injection due to the presence of neutralizing antibodies. However, in this study, there is no evidence that the efficacy of this drug as measured by an increase in priming threshold was compromised by any immune response to the first mAb injection. This could be due to the route of administration. Intravenous injections of large doses of proteins do not reliably induce immune responses. In fact, there is evidence for an inverse relationship between dose and immunogenicity, and an immune response is much more likely when the drug is administered intramuscularly or subcutaneously (Hwang and Foote, 2005). Since this is a humanized antibody, a more pronounced immune response might be expected in rats than humans. Overall, these results demonstrate that h2E2 does not lose efficacy following multiple injections.

In summary, the anti-cocaine mAb h2E2 greatly increased the amount of cocaine needed to reinstate self-administration behavior. This increase in priming threshold is accompanied by only a small decrease in inter-injection intervals. These effects may predict that h2E2 would be clinically efficacious in preventing cocaine relapse in cocaine abusers, and that repeated

treatments will be a viable option to allow for effective antibody concentrations to be maintained for long periods of time.

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Highlights

- The effects of two infusions of h2E2 on self-administration behavior were tested
- h2E2 increased the cocaine priming threshold following both injections
- h2E2 is a lead candidate for development as an immunotherapy for cocaine abuse

Fig. 1.

The effects of h2E2 on cocaine priming threshold. Note that plots are all shown on a linear scale for clarity. Each symbol represents the mean $\pm$ SEM priming threshold of 6 rats prior to day 35, and 5 rats after day 35. The straight lines represent the best fit of linear regression analyses for baseline for 6 rats before the day 35 and for 5 rats after that. Dashed lines represent the SEM for the regression. Star symbols represent means with a p -value less than α generated from paired t-tests with a false discovery correction. Arrows indicate h2E2 infusion.

Fig. 2.

Inter-injection intervals at a cocaine unit dose of 0.3 $\mu\text{mol/kg}$ (Panel A) and 3 $\mu\text{mol/kg}$ (Panel B) during maintained self-administration. Each symbol represents the mean $\pm$ SEM inter-injection intervals of baseline for 6 rats prior to day 35, and 5 rats afterwards. Dashed lines represent the SEM for the regression. The straight lines represent the best fit of linear regression analyses for 6 rats before the day 35 and for 5 rats after that. Star symbols represent means with a p -value less than α generated from paired t-tests with a false discovery correction. Arrows indicate h2E2 infusion.