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Clinical Potential of a Rationally Engineered Enzyme for Treatment of Cocaine Dependence: Long-lasting blocking of the psychostimulant, discriminative stimulus, and reinforcing effects of cocaine

Ting Zhang², Huimei Wei², Jing Deng², Fang Zheng^{1,2}, Chang-Guo Zhan^{1,2}

¹Molecular Modeling and Biopharmaceutical Center, College of Pharmacy, University of Kentucky, 789 South Limestone Street, Lexington, KY 40536

²Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 789 South Limestone Street, Lexington, KY 40536

Abstract

It is a grand challenge to develop a truly effective treatment of substance use disorder (SUD), particularly for cocaine and other drugs without an FDA-approved treatment available, because a truly effective therapy must effectively block the drug's physiological and reinforcing effects during the entire period of treatment in order to achieve the long-time abstinence required by the FDA. Whether a biologic, such as monoclonal antibody, vaccine, or therapeutic enzyme, can be truly effective for SUD treatment or not has been the subject of extensive debate. The main debate question is whether a biologic, particularly an exogenous enzyme, can effectively block the drug's reinforcing effect. In this report, we demonstrate that a modest dose of a recently redesigned long-acting cocaine hydrolase, Coch3-Fc(M6), can be used to effectively block the psychostimulant, discriminative stimulus, and reinforcing effects of cocaine for a sufficiently long period of time. For example, a dose of 3 mg/kg Coch3-Fc(M6) completely blocked the discriminative stimulus and reinforcing effects for 24/25 days and continued to significantly attenuate/decrease the cocaine effects for at least 29 days in rats. All the animal data consistently suggest that the long-acting cocaine hydrolase is a truly promising candidate of enzyme therapy for treatment of cocaine use disorder.

Correspondence to: Chang-Guo Zhan, Ph.D., Director, Molecular Modeling and Biopharmaceutical Center (MMBC), Director, Chemoinformatics and Drug Design Core of CPRI, Endowed College of Pharmacy Professor in Pharmaceutical Sciences, Professor, Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 789 South Limestone Street, Lexington, KY 40536, Phone: 859-323-3943, FAX: 859-257-7585, zhan@uky.edu.

Author Credit Statement

Ting Zhang: Methodology, Investigation, Formal analysis, and Visualization

Huimei Wei: Investigation

Jing Deng: Investigation

Fang Zheng: Conceptualization, Methodology, Funding acquisition, Writing- Reviewing and Editing

Chang-Guo Zhan: Conceptualization, Methodology, Funding acquisition, Writing-Reviewing and Editing

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Declaration of completing interest

None.

Introduction

Cocaine is recognized as one of the most reinforcing drugs of abuse without an FDA-approved pharmacotherapy specific for cocaine use disorder (CUD) – cocaine dependence or overdose (Landry, Zhao et al. 1993, Ersche, Jones et al. 2012, Milton and Everitt 2012, Wei, Shang et al. 2020, Wei, Zhang et al. 2020). Classical pharmacological approach to therapeutic treatment of CUD aims to block physiological effects of cocaine in the brain (Gorelick, Gardner et al. 2004). However, it would be extremely challenging for utilizing a classical pharmacological approach to directly block the physiological effects of cocaine without affecting the normal function of the brain (Huang, Gu et al. 2009, Yuan, Huang et al. 2015, Yuan, Huang et al. 2016). Aspiration to meet the unmet need has led to exploring alternative strategies using a biologic, such as a monoclonal antibody, vaccine, or catabolic enzyme, that aims to interact with cocaine itself (Zheng and Zhan 2009, Zheng and Zhan 2011, Zheng and Zhan 2012, Skolnick, White et al. 2015).

Compared to a cocaine antibody capable of stoichiometrically binding with cocaine, a catabolic enzyme of cocaine would be more efficient as each enzyme molecule can convert multiple cocaine molecules to physiologically inactive metabolites. The actual catalytic efficiency ($k_{\text{cat}}/K_{\text{M}}$) of an enzyme against cocaine is determined by the catalytic rate constant (k_{cat}) and Michaelis-Menten constant (K_{M}). Hydrolysis of cocaine at the benzyl ester group by butyrylcholinesterase (BChE) to produce physiologically inactive metabolites in plasma is known as the most favorable metabolic pathway of cocaine (Zhan, Zheng et al. 2003, Zhan, Deng et al. 2005, Gorelick 2008, Zheng, Yang et al. 2010, Chen, Zheng et al. 2017), although the catalytic efficiency ($k_{\text{cat}}/K_{\text{M}}$) of wild-type human BChE against naturally occurring (–)-cocaine is too low ($k_{\text{cat}} = 4.1 \text{ min}^{-1}$ and $K_{\text{M}} = 4.5 \text{ }\mu\text{M}$) (Sun, Pang et al. 2002). It was highly desirable to develop a mutant of human BChE with considerably improved catalytic efficiency against (–)-cocaine for development of an enzyme therapy to treat cocaine use disorders (Zheng and Zhan 2008, Zheng and Zhan 2012). For convenience, throughout this report, cocaine always refers to the naturally occurring (–)-cocaine, unless stated explicitly otherwise.

Indeed, by using our previously developed unique structure-and-mechanism-based computational enzyme redesign approach, we successfully designed and discovered human BChE mutants, known as cocaine hydrolases (CocHs), with considerably improved catalytic efficiency against cocaine (Pan, Gao et al. 2005, Zheng, Yang et al. 2008, Xue, Ko et al. 2011, Zheng, Xue et al. 2014) and its physiologically active metabolites (Hou, Xue et al. 2013, Hou, Zhan et al. 2014, Zhan, Hou et al. 2014, Chen, Zheng et al. 2016, Zheng, Chen et al. 2020). Our first CocH (CocH1 which is the A199S/S287G/A328W/Y332G mutant of human BChE) (Pan, Gao et al. 2005, Yang, Xue et al. 2010, Zhang, Zheng et al. 2017, Zheng, Zhou et al. 2018) was fused with human serum albumin (HSA) (Brimijoin, Gao et al. 2008) in clinical development for treatment of cocaine dependence. This HSA-fused protein is also known as Albu-CocH1, Albu-CocH, TV-1380, or Albu-BChE in literature (Brimijoin, Gao et al. 2008, Cohen-Barak, Wildeman et al. 2015, Shram, Cohen-Barak et al. 2015, Willyard 2015, Gilgun-Sherki, Eliaz et al. 2016, Shemesh-Darvish, Shinar et al. 2018). The safety of TV-1380 for use in humans has been confirmed favorably in multiple Phase I

clinical trials (Cohen-Barak, Wildeman et al. 2015, Shram, Cohen-Barak et al. 2015). During a Phase II clinical trial in cocaine-dependent patients (Gilgun-Sherki, Eliaz et al. 2016), TV-1380 at a once-weekly dosing schedule (up to 300 mg per dose) significantly and dose-dependently decreased cocaine intake in the treatment groups compared to the placebo group, and non-significantly increased the FDA-defined cocaine abstinence rate. So, the biological half-life ($t_{1/2}$ = 8 hours in rats and 43–77 hours in humans) of TV-1380 was not sufficiently long for the practical cocaine dependence treatment to effectively keep abstinence from cocaine using TV-1380 with the desired once-weekly dosing schedule (Gilgun-Sherki, Eliaz et al. 2016). The clinical outcomes are not surprising, because they are consistent with the corresponding preclinical data (Schindler, Justinova et al. 2013), *i.e.* administration of 5 mg/kg TV-1380 was only able to decrease cocaine (30 µg/kg/infusion) self-administration in monkeys for three days, and it was unable to completely block the cocaine self-administration in monkeys even on Day 1.

Possible option to improve the clinical outcomes of TV-1380 in treatment of cocaine dependence was to increase the TV-1380 dose, which would increase the cost for producing each dose of the enzyme, or increase the dosing frequency (*e.g.* twice per week). For the later, unfortunately, an injection product for SUD treatment would be unacceptable if it had to be administered more frequently than once weekly, based on a survey of treatment providers (Gilgun-Sherki, Eliaz et al. 2016). For this reason, the clinical development program with TV-1380 was terminated (Gilgun-Sherki, Eliaz et al. 2016).

To solve the dosing frequency problem, we have designed and discovered a long-acting form of CocH by using human immunoglobulin G1 (IgG1) Fc fragment, instead of HSA, as an alternative carrier protein to produce Fc-fused CocH protein, denoted as CocH-Fc, with a significantly improved biological half-life (Chen, Xue et al. 2016, Chen, Deng et al. 2019). Through further structure-based computational design, our recently designed CocH-Fc mutant, known as CocH3-Fc(M6), has a markedly prolonged biological half-life ($t_{1/2}$ = ~206±7 hours) in rats (Zheng, Chen et al. 2020). It is interesting to know whether this long-acting enzyme, *i.e.* CocH3-Fc(M6), can effectively block the discriminative stimulus and reinforcing effects of cocaine for a sufficiently long period of time.

The focus of the present study was to determine the length of time a single injection of enzyme CocH3-Fc(M6) may be active in the body. Here we demonstrate for the first time that CocH3-Fc(M6) is capable of completely blocking the discriminative stimulus and reinforcing effects of cocaine in rats for a desirably long period of time (weeks).

Materials and Methods

Materials.

The purified protein of CocH3-Fc(M6) was prepared in our previous study (Zheng, Chen et al. 2020). The protein was stored in –80 °C before use. Male Sprague-Darley rats (200–275 g) were ordered from Harlan (Harlan, Indianapolis, IN). All the animal experiments were conducted in our animal laboratories within the University of Kentucky's Division of Laboratory Animal Resources (DLAR) facility (PHS assurance number A3336–01; USDA number 61-R-0002; AAALAC, Intl. Unit # 13). The animal procedures used in this project

were approved by the Institutional Animal Care and Use Committee (IACUC). Veterinary care and animal husbandry were provided and supervised by the staff of the DLAR facility. All animals were housed in clean, adequately-sized, stainless-steel cages at 21–22 °C and were allowed *ad libitum* access to food and water for 1 week before any experiments. They were monitored daily by the study staff and by members of the veterinary staff for general health to detect signs of discomfort due to testing and/or the administration of drugs.

Combined locomotor activity testing and PK analysis.

A group of 8 rats (n=8) were administered intravenously (IV) with a dose of 3 mg/kg CocH3-Fc(M6), followed by daily blood sampling and locomotor activity testing on Days –2 (baseline locomotor activity), 0, 1, 3, 5, 7, 9, 11, 18, 25, and 32 after the enzyme administration. Blood samples were taken from saphenous vein puncture using a needle. Each time, approximately 50–80 µL blood was collected into a heparin-coated capillary tube. For comparison in the locomotor activity testing, another group of 8 rats (n=8) were administered IV with saline, instead of the enzyme. On the locomotor activity testing day, blood was collected right after the locomotor activity testing. Collected blood samples were centrifuged for 15 min at a speed of 5000 *g* to separate the plasma, and the plasma was kept at 4 °C before analysis. A sensitive radiometric assay (Zheng, Xue et al. 2014, Chen, Xue et al. 2016) with 100 µM (–)-cocaine was used to measure the enzyme activity (*ex vivo* pharmacodynamics) in plasma and convert the *ex vivo* cocaine hydrolysis activity to the concentration of the enzyme: $[E] = V_{\max}/k_{\text{cat}}$. The time-dependent active enzyme concentrations ($[E]_t$) were fitted to a well-known double-exponential equation (Lockridge, Schopfer et al. 2005) by using the GraphPad Prism 7: $([E]_t = Ae^{-k_1t} + Be^{-k_2t})$ which accounts for both the enzyme distribution process (the fast phase, associated with k_1) and elimination process (the slow phase, associated with k_2). The half-life ($t_{1/2}$) associated with the enzyme elimination rate constant k_2 is known as the elimination half-life or biological half-life.

For each locomotor activity test, 20 mg/kg cocaine was administered intraperitoneally (IP), followed by the locomotor activity monitoring. The locomotor activity was monitored in high density, non-porous plastic chambers measuring 50 cm (L) × 50 cm (W) × 38 cm (H) in a light- and sound-attenuating behavioral test enclosure (San Diego Instruments, San Diego, CA). Cumulative horizontal distance traveled was recorded by EthoVision XT video tracking system (Noldus Information Technology, Wageningen, Netherlands) to represent the locomotor activity. The hyperactivity was reflected by the increased horizontal distance traveled.

Drug discrimination tests in rats.

A group of rats (n=6) were trained to discriminate both 10 mg/kg cocaine (IP) and 0.56 mg/kg methamphetamine (METH, IP) from saline. Drug discrimination sessions were conducted in operant conditioning chambers with an interior size of 30.5 cm (L) × 24.1 cm (W) × 21 cm (H) placed in a sound attenuating cubicle (Med Associates, Fairfax, VT). Each chamber was equipped with two retractable levers and a pellet dispenser which delivered 45 mg sucrose pellets (Bio-Serv, Frenchtown, NJ) into a pellet cup between the two levers. In

the chamber, a house light was mounted on the wall facing the levers and pellet cup and a white light was located above each of the levers.

Rats are maintained at 80% of their free-feeding weights for the period of the study. They were trained to respond for sucrose pellets under a fixed ratio-1 with a 5-second time out (TO) schedule (FR1TO5) with only one lever available (left or right during alternating sessions) initially. The fixed ratio (FR) was gradually increased to FR10TO5. Once rats consistently earned 10 pellets in 10-min sessions, they were trained to discriminate both 10 mg/kg (IP) cocaine and 0.56 mg/kg (IP) ($\pm$)-methamphetamine (METH) from saline using a double-alternation (SSCCSSCC...), two-lever discrimination procedure described as the standard protocol (Solinas, Panlilio et al. 2006) and in literature (Collins, Narasimhan et al. 2012). The position (right/left) of the drug (cocaine or METH)-appropriate lever was counterbalanced across rats.

During each training session, following drug (cocaine or METH) or saline administration, rats were placed into operant chambers, illuminated by house light, for a 15-min pretreatment period. The start of the session was signaled by the EXT of the house light, insertion of the levers, and the illumination of the white light above each of the levers. Completion of 10 presses (FR10) on the injection-appropriate lever resulted in sucrose pellet delivery followed by a 5-second time-out (TO5) during which the white lights above the levers were extinguished. Responding on the alternate lever was recorded but had no scheduled consequence. The session was terminated after 10 pellets had been earned or 10 min had been elapsed, whichever came first. When session ended, levers were retracted, white lights were extinguished, and the house light was illuminated.

After acquisition of discrimination and collection of stable baseline responses, for each rat, a single dose (3 mg/kg) of protein Coch3-Fc(M6) was administered (IV), followed by daily cocaine (10 mg/kg) discrimination tests within 40 days (Days 1 to 40). In addition, on Days 1, 3, 6, 8, 10, 13, 15, 17, 20, 22, 24, 26, 28, 30, 32, 34, 36, and 39, the cocaine (10 mg/kg) discrimination tests were also followed by METH (0.56 mg/kg) discrimination tests for comparison.

Cocaine self-administration tests in rats.

Intravenous self-administration (IVSA) of cocaine was carried out to evaluate the reinforcing effect of cocaine in rats. After one-week acclimation period in the new animal facility, rats were introduced to the operant conditioning chambers for food-reinforced lever press training for a 10 min session daily. Once rats were able to earn 10 food pellets within 10 min under FR5 on 3 consecutive days, they were considered as well-trained and subjected to the catheter implantation surgery.

All the rats that have passed the initial food training were implanted with polyurethane catheters (C30PU-RFV1418, Instech Laboratories, PA) in the left femoral vein under isoflurane anesthesia according to the method described in literature (Thrivikraman, Huot et al. 2002). The opposite end of the catheter was passed under the skin to the rat's back exteriorized in the scapular attached to a rat VAH harness (VAH95AB, Instech Laboratories, PA). Rats were allowed to recover for 6–7 days before the experiment. Catheters were

flushed daily with 0.2 ml of heparinized saline (100 U/ml) before the start, and after the completion of operant sessions.

All self-administration sessions were conducted in operant conditioning chambers as used in the aforementioned drug discrimination tests. Each chamber was equipped with two retractable levers. In the chamber, a house light was mounted on the wall facing the levers and a white light was located above each of the levers. A VTA spring tether (VAH95T, Instech Laboratories, PA) was held above the chamber by a counterbalanced arm to connecting the syringe and external port on the harness. The syringe pumps (PMH-100; Med Associates) were used to deliver solutions under the control of the Med-PC IV Software (SOF-735, Med Associates).

Rats were initially trained to press one lever (defined as the active level) for infusion of 0.4 mg/kg/infusion (unit dose) cocaine during a 2-hour session daily under an FR1TO5 schedule of reinforcement with the active lever counterbalanced across rats. At the beginning of the session, the house light and fan were turned on. Illumination of the white light above the active lever signaled drug availability. The press on the active lever resulted in unit dose of cocaine infusion. During the 5-s TO period, the white light located on the active level was turned off and responses were recorded but had no scheduled consequence. Once they were able to acquire 20 infusions per session, the ratio requirement was gradually increased (1, 3, 5) until rats were responding under the FR5TO5 schedule of reinforcement. After the cocaine intake under this schedule was stabilized, rats were allowed to have access to cocaine for at least 7 sessions as maintenance training.

Upon stabilization of responding for cocaine intake, one group of 8 rats (n=8) were tested in a pilot IVSA study at various unit doses of cocaine (0, 0.032, 0.1, 0.32, and 1.0 mg/kg/infusion) in the ascending order with each IVSA session lasting for 30 min; the five 30-min sessions associated with the five unit doses were carried out on the same day. We elected to use the very short, 30-min cocaine self-administration sessions for a couple of reasons. First, a previously reported cocaine self-administration study (Mantsch, Li et al. 2007) also used the 30-min sessions, and another previously reported cocaine self-administration study (Collins, Narasimhan et al. 2012) used even shorter (25-min) sessions; these short sessions all showed stable responses in rats. In addition, our own pilot IVSA study also indicated that the responses to various unit doses (0, 0.032, 0.1, 0.32, and 1.0 mg/kg/infusion) in the ascending order with each IVSA session lasting for 30 min can be repeated from day to day; in other words, the cocaine IVSA experience in these sessions and the earlier cocaine IVSA sessions using 0.4 mg/kg/infusion did not affect the cocaine IVSA during subsequent days.

Two other groups of rats (n=8 for saline control group and n=6 for the enzyme treatment group) were used for cocaine IVSA tests at a unit dose of 0.1 mg/kg/infusion after they were trained to stably self-administer cocaine (0.1 mg/kg/infusion) in 30-min sessions. We elected to use the 30-min sessions for the further IVSA tests because we saw the stable responses in the above-mentioned pilot study using the 30-min sessions. The rats were administered (IV) with 3 mg/kg CocH3-Fc(M6) in the treatment group or vehicle (saline) in the control group, followed by cocaine (0.1 mg/kg/infusion) IVSA sessions on Days 0, 1, 3, 4, 6, 7, 8, 10, 11,

13, 14, 15, 17, 18, 20, 21, 22, 24, 25, 27, 28, and 29 after the CocH3-Fc(M6) (enzyme treatment group) or saline (saline control group) administration.

Additional experiments to test the effects of CocH3-Fc(M6) on the food self-administration and locomotor activity in rats.

An additional group of 8 rats ($n=8$) were trained to earn food pellets in 40-min sessions of food self-administration under FR1. The maximum number of rewards (food pellets) allowed in each session was 40. In other words, for each rat, the session was terminated after the maximum of 40 rewards had been earned or 40 min had been elapsed, whichever came first. In addition, the same group of rats were also tested for their locomotor activity during a session of three hours, including one hour (acclimation) before, and two hours after, IP administration of saline. Upon stabilization of the food self-administration, the rats were administered (IV) with 3 mg/kg CocH3-Fc(M6), followed by both the food self-administration sessions and locomotor activity sessions on the same days (Days 1, 3, 5, 7, and 9). Each locomotor activity session lasted three hours, including one hour (acclimation) before, and two hours after, IP administration of saline.

Statistical analysis.

The comparison in animal data between different groups of rats was performed by two-way (treatment $\times$ time) analysis of variance (ANOVA) with *post hoc* tests (with repeated measurements over time). The in-group comparison was carried out by one-way (time) ANOVA with *post hoc* tests (with repeated measurements over time). Comparisons with baselines were performed using the paired *t*-test. All statistical analyses were performed using the GraphPad Prism 7.0 software (GraphPad Software, La Jolla, CA).

Results

Impact on the psychostimulant effect of cocaine and the threshold plasma concentration of the enzyme for completely blocking cocaine-induced hyperactivity.

During the combined locomotor activity testing and PK analysis, rats were administered intravenously (IV) with a dose (3 mg/kg) of CocH3-Fc(M6), followed by daily blood sampling for determining the PK profile and for locomotor activity testing on Days -2 (baseline locomotor activity), 0, 1, 3, 5, 7, 9, 11, 18, 25, and 32 after the enzyme administration. For each locomotor activity test, 20 mg/kg cocaine was administered intraperitoneally (IP), followed by the locomotor activity monitoring. Here, the psychostimulant effect of cocaine is represented by cocaine-induced hyperactivity (the increase of locomotor activity from the baseline). As shown in Figure 1A, 3 mg/kg CocH3-Fc(M6) had a biological half-life ($t_{1/2} = \sim 206$ hours) which is consistent with previously determined biological half-life ($t_{1/2} = \sim 206$ hours) of CocH3-Fc(M6) at a much lower dose (0.075 mg/kg) (Zheng, Chen et al. 2020). Indeed, the biological half-life of ~ 206 hours was long enough for a single dose (3 mg/kg) of CocH3-Fc(M6) to completely block cocaine-induced hyperactivity for at least 18 days and still considerably attenuate cocaine-induced hyperactivity on Days 25 and 32 (Figure 1B). According to the PK profile depicted in Figure 1A, the average plasma concentrations of CocH3-Fc(M6) on Days 18, 25, and 32 were 4.91, 2.82, and 1.56 mg/L, respectively, suggesting that the threshold plasma concentration of

Coch3-Fc(M6) for completely blocking 20 mg/kg cocaine-induced hyperactivity in rats should be between 4.91 mg/L (Day 18) and 2.82 mg/L (Day 25).

Impact on the discriminative stimulus effect of cocaine.

To evaluate the impact of Coch3-Fc(M6) on discriminative stimulus effect of cocaine, a group of rats ($n=6$) were trained to discriminate both 10 mg/kg cocaine (IP) and 0.56 mg/kg METH (IP) from saline. After acquisition of discrimination and collection of stable baseline responses, a single dose (3 mg/kg) of Coch3-Fc(M6) was administered (IV), followed by daily cocaine (10 mg/kg) discrimination tests and METH (0.56 mg/kg) discrimination tests within 40 days after the Coch3-Fc(M6) administration. As shown in Figure 2A, a single dose (3 mg/kg) of Coch3-Fc(M6) completely blocked the discriminative stimulus effect of 10 mg/kg cocaine for 25 days, and still significantly attenuated the discriminative stimulus effect of 10 mg/kg cocaine on Days 26 to 35, without significantly affecting the discriminative stimulus effect of 0.56 mg/kg METH (Figure 2A) and without significantly decreasing the response rates (Figure 2B). The drug discrimination data in Figure 2 together with the PK data in Figure 1A suggest that the threshold plasma concentration of Coch3-Fc(M6) for completely blocking the discriminative stimulus effect of 10 mg/kg cocaine in rats should be between 2.82 mg/L on Day 25 and 2.64 mg/kg on Day 26. When the plasma concentration of Coch3-Fc(M6), denoted as [E] for convenience, is 2.82 mg/L or higher ($[E] \geq 2.82$ mg/L), the enzyme can completely block the discriminative stimulus effect of 10 mg/kg cocaine. When $[E] = 2.64$ mg/L, the enzyme cannot completely block, but can still significantly attenuate, the discriminative stimulus effect of 10 mg/kg cocaine.

Impact on the reinforcing effects of cocaine.

The reinforcing effect of cocaine was tested through intravenous self-administration (IVSA) of cocaine. Three groups of rats were trained to eventually self-administer cocaine under a fixed ratio (FR) schedule, FR5, requiring 5 operant responses (*via* the active lever) to dispense a unit dose of cocaine *via* the IV catheter. The first group of rats ($n=8$) were used to carry out a pilot IVSA study using various unit doses. Upon stabilization of responding, this group of rats were tested in IVSA at various unit doses of cocaine (0, 0.032, 0.1, 0.32, and 1.0 mg/kg/infusion) with each IVSA session lasting for 30 min. As seen in Figure 3A, the average number of cocaine infusions during the 30-min session increased when the unit dose increased from 0 (saline as control) to 0.032 and 0.1 mg/kg/infusion. However, the average number of cocaine infusions decreased with further increasing the unit dose from 0.1 to 0.32 and 1 mg/kg/infusion. The average number of cocaine infusions reached the maximum (23.3) at unit dose of 0.1 mg/kg/infusion.

So, further cocaine IVSA tests were performed at the unit dose of 0.1 mg/kg/infusion in other two groups of rats after they were trained to stably self-administer cocaine (0.1 mg/kg/infusion). After the baseline IVSA data were collected, the rats were administered (IV) with 3 mg/kg Coch3-Fc(M6) in the treatment group ($n=6$) or vehicle (saline) in the control group ($n=8$), followed by cocaine (0.1 mg/kg/infusion) IVSA sessions on Days 0, 1, 3, 4, 6, 7, 8, 10, 11, 13, 14, 15, 17, 18, 20, 21, 22, 24, 25, 27, 28, and 29 after the Coch3-Fc(M6) administration (Figure 3B). As shown in Figure 3B, the rats in the saline control group had the stable response in all the cocaine (0.1 mg/kg/infusion) IVSA sessions. For the rats in the

treatment group, a single dose of 3 mg/kg Coch3-Fc(M6) completely blocked cocaine (0.1 mg/kg/infusion) self-administration for 24 days and still significantly decreased cocaine (0.1 mg/kg/infusion) self-administration on Days 25 to 29 in comparison with the rats in the control group (see Figure 3B). The IVSA data in Figure 3B together with the PK data in Figure 1A suggest that the threshold plasma [E] of Coch3-Fc(M6) for completely blocking the reinforcing effect of 0.1 mg/kg/infusion cocaine should be between 3.04 mg/kg on Day 24 and 2.82 mg/L on Day 25. When [E] = 3.04 mg/L, the enzyme can completely block the reinforcing effect of 0.1 mg/kg/infusion cocaine. When [E] = 2.82 mg/L, the enzyme cannot completely block, but can still significantly decrease the reinforcing effect of 0.1 mg/kg/infusion cocaine.

Effects of Coch3-Fc(M6) on the food self-administration and locomotor activity in rats.

A reasonable question was whether animal behavior (locomotion and lever pressing) was decreased by Coch3-Fc(M6). According to the drug discrimination data in Figure 2, Coch3-Fc(M6) did not decrease the response rate (Figure 2B) or affect the METH discrimination from saline (Figure 2A), suggesting that Coch3-Fc(M6) itself did not change the animal behavior. We also carried out additional food self-administration and locomotor activity tests in another group of 8 rats (without cocaine administration) to further examine the question. The obtained data are shown in Figure 4, showing no significant change on the number of rewards (Figure 4A) or response rate (Figure 4B) in the food self-administration after IV administration of 3 mg/kg Coch3-Fc(M6) compared to the corresponding pre-dose baseline data. Meanwhile, according to the data in Figure 4C, IV administration of 3 mg/kg Coch3-Fc(M6) did not significantly decrease the locomotor activity of rats compared to the pre-dose baseline locomotor activity. These additional food self-administration and locomotor activity data further confirmed that enzyme Coch3-Fc(M6) itself had no significant effects on the animal behavior.

Discussion

It is a grand challenge to develop a truly effective SUD treatment, particularly for cocaine and other drugs (*e.g.* amphetamine and methamphetamine) without an FDA-approved treatment available. This is because a truly effective therapy may have to completely block the drug's physiological and reinforcing effects during the entire period of treatment in order to achieve the long-time abstinence required by the FDA. Specifically, for cocaine, most therapeutic discovery and development efforts have been focused on identifying therapeutic candidates capable of attenuating the psychostimulant, discriminative stimulus, and/or reinforcing effects of cocaine, with the goal to decrease cocaine intake. However, merely decreasing cocaine intake cannot meet the FDA-defined clinical endpoint. The primary endpoint acceptable to the FDA is the proportion of participants achieving abstinence from cocaine (no cocaine intake at all) during the last three weeks of the cocaine dependence treatment period (which must be at least 12 weeks) based on daily self-report of "no use" confirmed by urine testing (Gilgun-Sherki, Eliaz et al. 2016). The minimum 12-week treatment period requirement is based on the understanding that exposure to cocaine will increase the cell surface DAT expression for a long time, as normalization of dopaminergic function is generally a slow process (Schmitt and Reith 2010). Due to the increase of surface

dopamine transporter (DAT) expression (because of DAT trafficking induced by cocaine), there are less dopamine molecules available in the synapse for signaling, which may contribute to the drug seeking or craving. Nevertheless, according to a monkey study (Beveridge, Smith et al. 2009), following 90 days of abstinence from chronic cocaine self-administration, the levels and DAT and dopamine receptors were not different from the control values of the monkeys without cocaine exposure. So, abstinence from cocaine for about 90 days or 12 weeks is expected to be enough for the dopaminergic function normalization and, thus, for the cocaine dependence treatment.

In fact, numerous therapeutic candidates capable of *attenuating* (rather than completely blocking) the discriminative stimulus or reinforcing effects of cocaine have been developed (Czoty, Stoops et al. 2016) and evaluated in clinical trials, but none has been proven effective to achieve the FDA-defined abstinence in cocaine dependence treatment. Particularly for TV-1380, in previous cocaine self-administration study (Schindler, Justinova et al. 2013), a dose of 5 mg/kg TV-1380 only decreased cocaine (30 µg/kg/infusion) self-administration in monkeys for 3 days, and it did not completely block the cocaine self-administration in the monkeys after one day. Similar to the outcomes of the cocaine (30 µg/kg/infusion) self-administration study in monkeys, in the 12-week Phase II clinical trial with a once-weekly dosing schedule (up to 300 mg TV-1380 per dose) (Gilgun-Sherki, Eliaz et al. 2016), TV-1380 indeed significantly and dose-dependently decreased cocaine intake in the treatment groups compared to the placebo group (Gilgun-Sherki, Eliaz et al. 2016), but it non-significantly increased the proportion of subjects achieving the FDA-defined abstinence from cocaine. Hence, an ideal approach to development of a truly effective cocaine dependence treatment is to identify a therapeutic candidate capable of completely blocking the psychostimulant, discriminative stimulus, and reinforcing effects of cocaine for a sufficiently long period of time.

As noted above, a single dose of 3 mg/kg Coch3-Fc(M6) completely blocked all of the above-mentioned cocaine effects in rats for a long period of time, including the discriminative stimulus and reinforcing effects for 24/25 days, and continued to significantly attenuate/decrease the cocaine effects for at least 29 days. This is the first demonstration that one-time administration of an exogenous enzyme is capable of completely blocking all of the psychostimulant, discriminative stimulus, and reinforcing effects of cocaine for a long period of time (days). So, in the presence of a sufficiently high concentration of enzyme Coch3-Fc(M6) in plasma, the enzyme can rapidly convert cocaine to physiologically inactive metabolites whenever a subject takes cocaine again. As a result, cocaine would be unable to produce any psychostimulant, discriminative stimulus, or reinforcing effect, like saline, to ensure achieving the FDA-defined abstinence from cocaine.

Further, it should be noted that, for an Fc-fused protein drug such as abatacept (EMA), its biological half-life in humans (*e.g.* $t_{1/2}$ = 12–23 days for abatacept) is usually longer than that (*e.g.* $t_{1/2}$ = 3–6 days for abatacept) in rats by about 3 to 4-fold. For this reason, the change in the biological half-life of Coch3-Fc(M6) from rats to humans could be similar to that of abatacept. If this is the case, the duration of the 3 mg/kg Coch3-Fc(M6) action in humans might be markedly longer than the 24/25 days observed in rats and, hence, the

anticipated 12-week period of cocaine dependence treatment may need only three doses (once every four weeks) of the CocH3-Fc(M6) protein.

Concerning the general concept of SUD treatment, whether a biologic, such as monoclonal antibody, vaccine, or therapeutic enzyme, can be truly effective for SUD treatment has led to extensive debate. The main debate question is whether a biologic can effectively block the drug's reinforcing effect. This crucial question has been addressed through our cocaine self-administration tests in the presence of CocH3-Fc(M6). Our animal data indicate that a therapeutic enzyme must be both highly efficient and long acting enough to be truly effective for SUD treatment, and this is achievable. The similar therapeutic strategy may be used to develop efficient enzyme therapies for other drugs of abuse.

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Highlights

- There is no FDA-approved medication specific for cocaine overdose or dependence
- It is challenging to block effects of cocaine without affecting normal function of brain
- Accelerating cocaine hydrolysis is recognized as a promising therapeutic strategy
- An enzyme is capable of long-lasting blocking of cocaine discrimination in rats
- The enzyme is capable of long-lasting blocking of cocaine self-administration in rats

Figure 1.

Pharmacokinetic (PK) profile of 3 mg/kg CochH3-Fc(M6) and its long-term effect on cocaine-induced hyperactivity after IV administration of CochH3-Fc(M6) in eight rats (n=8) in comparison with the saline control group (n=8). IV administration of 3 mg/kg CochH3-Fc(M6) was followed by daily blood sampling (treatment group only) for determining the PK profile (panel A) and locomotor activity testing on Days -2 (baseline locomotor activity), 0, 1, 3, 5, 7, 9, 11, 18, 25, and 32 after the CochH3-Fc(M6) administration. For each locomotor activity test, 20 mg/kg cocaine was administered IP (panel B). The statistical differences between the CochH3-Fc(M6) treatment group and control group were analyzed by two-way ANOVA ($F(9, 126) = 12.58, p < 0.0001$) with *post hoc* tests: **** $p < 0.0001$. Within the CochH3-Fc(M6) treatment group, the statistical differences for the locomotor activity on

different days *vs* the saline baseline were analyzed by one-way ANOVA ($F(10, 77) = 8.381$, $p < 0.0001$) with *post hoc* tests: # $p < 0.05$.

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Figure 2.

Long-term influence of Coch3-Fc(M6) on the discriminative stimulus effects of cocaine and methamphetamine (METH) in a group of six rats ($n=6$). A single dose of Coch3-Fc(M6) (3 mg/kg, IV) was followed by multiple sessions of cocaine (10 mg/kg, IP) and METH (0.56 mg/kg, IP) discrimination testing within 40 days after the Coch3-Fc(M6) administration. The statistical differences between different treatment conditions were analyzed by one-way ANOVA ($F(2, 65) = 37.18$, $p < 0.0001$) with *post hoc* analysis: $p = 0.2039$ for the cocaine group vs the saline group; $p < 0.0001$ for the cocaine group vs the METH group. The differences between the cocaine group on different days and the saline baseline were analyzed by one-way ANOVA ($F(41, 222) = 19.01$, $p < 0.0001$) with *post hoc* tests: $p > 0.05$

(Days 0 to 25) and $p < 0.05$ (Day 26 and thereafter) for the cocaine group vs the saline baseline.

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Figure 3.

Long-term influence of Coch3-Fc(M6) on the reinforcing effect of cocaine. (A) Dose-response curve of cocaine self-administration in rats ($n=8$). (B) A single dose of Coch3-Fc(M6) (3 mg/kg, IV, for the enzyme treatment group, $n=6$) or saline (for the control group, $n=8$) was followed by multiple sessions of cocaine (0.1 mg/kg/infusion) self-administration (FR5) testing within 29 days after the Coch3-Fc(M6) administration. The statistical differences between the Coch3-Fc(M6) treatment group and the control group were analyzed by two-way ANOVA ($F(28, 336) = 6.135, p < 0.0001$) with *post hoc* tests: **** $p < 0.0001$. Within the Coch3-Fc(M6) treatment group, the statistical differences between different days and the zero-response baseline were analyzed by one-way ANOVA ($F(21, 110) = 2.796, p = 0.0003$) with *post hoc* tests: # $p < 0.05$.

Figure 4.

Effects of CochH3-Fc(M6) on the food self-administration and locomotor activity in rats ($n=8$). Food self-administration and locomotor activity before (pre-dose) and after IV administration of 3 mg/kg CochH3-Fc(M6), showing no significant changes after the CochH3-Fc(M6) administration (according to the paired t -test): (A) number of rewards (food pellets) earned in each food self-administration session; (B) response rate in each food self-administration session; (C) total distance moved in two hours after IP administration of saline in each locomotor activity session.