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Enzyme-therapy approaches for the treatment of drug overdose and addiction

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Abstract

“Recent progress in the study of cocaine-metabolizing enzymes demonstrates that enzyme-therapy approaches using appropriately designed enzymes show promise for the treatment of drug overdose and addiction.”

Keywords

drug abuse; drug addiction; drug metabolism; drug overdose; rational drug design; therapeutic enzyme

Substance abuse and addiction are a major global medical and social problem [101]. Most abused substances are psychoactive drugs, such as cocaine, illicit opiates (opium, morphine and heroin), amphetamine-type stimulants (amphetamine, methamphetamine, methcathinone and related substances), ecstasy-group substances (3,4-methylenedioxymethamphetamine and its analogues) and cannabinoids (a class of compounds found in cannabis, also known as marijuana or marihuana). Some licit substances, including nicotine and alcohol, are also psychoactive compounds. Psychoactive drugs are able to cross the blood–brain barrier (BBB) and act primarily on the CNS to alter brain functions, which results in changes in perception, mood, consciousness, cognition and behavior [102]. It has been estimated that the total overall costs of substance abuse in the USA alone, including health- and crime-related costs as well as loss in productivity, are more than half a trillion dollars per year [102]. The cost estimate did not fully describe the breadth of deleterious public health and safety implications, including loss of lives, family disintegration, loss of employment, failure in school, domestic violence, child abuse and other crimes. The disastrous consequences of drug abuse and addiction have made the development of antidrug medication a high priority.

It is a great challenge to develop a truly effective therapeutic treatment for drug overdose and addiction. In general, the pharmacological treatment of drug overdose and addiction can

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be either pharmacodynamic or pharmacokinetic [1]. Most currently employed anti-addiction strategies use the classical pharmacodynamic approach, that is, developing small molecules that interact with one or more neuronal binding sites, with the goal of blocking or counteracting neuropharmacological actions of the drug. Thus, most currently used antidrug medications are direct or indirect agonists/antagonists of the drugs [2]. Advances in the neuroscience of drug abuse and dependence in the past decades have led to a deeper understanding of the molecular basis of drug dependence and addiction [3–8], which helps identify more appropriate targets for more promising pharmacodynamic agents. In fact, valuable pharmacodynamic agents have been developed for most, but not all, drugs of abuse. Due to the complex interrelations of neuronal circuits, it is difficult to accurately predict the actions of agonist/antagonist types of therapeutic candidates and design an agonist/antagonist without undesirable side effects within the CNS [2]. It is especially difficult to design an ideal pharmacodynamic agent for the treatment of cocaine overdose and abuse. As is well known, cocaine binds to dopamine transporter (DAT) and other transporters/receptors. In the primary target (DAT), the cocaine binding site overlaps with the dopamine binding site after initial cocaine binding with DAT [9–11]. Thus, it would be extremely difficult to develop an antagonist that can potentially block DAT–cocaine binding without affecting the normal DAT–dopamine interaction and downstream signal transduction. Hence, despite decades of effort, existing pharmacodynamic approaches to cocaine abuse treatment have not yet been proven successful [1,2]. There is still no US FDA-approved therapeutic agent specific for cocaine. Novel pharmacological approaches to the treatment of cocaine overdose and addiction are highly desirable.

The inherent difficulties in antagonizing a blocker like cocaine in the CNS have led to the development of pharmacokinetic approaches that aim to act directly on the drug itself to alter its distribution or accelerate its clearance [1]. Pharmacokinetic antagonism of a drug could be implemented by using a protein, such as an antibody, which binds tightly to the drug in such a way that the drug–antibody complex cannot cross the BBB [3]. The antibody could be provided with either active immunization (vaccine) [12–14] or passive immunity (monoclonal antibody produced in another host) [15,16]. The blocking action could also be implemented by administration of an appropriately designed enzyme [1,17], or a catalytic antibody (regarded as an artificial enzyme) [18] that not only binds but also accelerates drug metabolism, thereby freeing itself for further binding. After an enzyme molecule degrades a drug molecule and the metabolites leave the active site of the enzyme, the enzyme molecule can bind with and metabolize another drug molecule. Thus, an enzyme molecule can be used repeatedly, until all the drug molecules are metabolized.

The use of a protein-based pharmacokinetic approach (enzyme, antibody or vaccine) has a potential advantage over traditional pharmacodynamic approaches using a small-molecule compound, since a protein-based pharmacokinetic agent will not usually be expected to cross the BBB and reach the CNS. Therefore, an appropriately designed protein-based pharmacokinetic agent is not expected to block the normal functions of the transporters and receptors in the CNS. For this reason, a pharmacokinetic agent itself would have no direct pharmacodynamic action, such as abuse liability [1].

Now that a pharmacokinetic agent does not reach the CNS, one might ask whether an exogenous enzyme or monoclonal antibody can also clear up the drug molecules that have already reached the CNS, before the enzyme or antibody is injected. It is well-known that a small-molecule drug of abuse, such as cocaine, can cross the BBB back and forth very quickly to reach an equilibrium between the blood and brain, because usually the drug effects can be observed immediately after administration of the drug. Hence, when the concentration of the free drug in the blood decreases, drug molecules in the brain will cross the BBB and return to the blood.

Within the protein-based pharmacokinetic approaches, the administration of an exogenous enzyme or monoclonal antibody would immediately interact with cocaine, and would not require an immune response to be effective, as opposed to the active immunization (vaccine approach). The use of an exogenous enzyme has a major advantage over the antibody or vaccine approaches, in that an antibody binds stoichiometrically with a drug. Even if the binding affinity of an antibody with a drug were infinitely high, an antibody molecule could only bind with one drug molecule. The antibody or vaccine approach is expected to work well when the drug is at low concentration in the body. However, when the drug concentration is high, it would be very difficult to have an equal molar concentration of its antibody in the body to bind with all drug molecules, as the molecular weight of an antibody is usually many hundredfold larger than that of a drug of abuse. So, in the case of drug overdose, the antibody would be saturated by the drug such that most of the drug molecules are still free for their actions. The molecular weight of a drug-metabolizing enzyme is usually comparable to that of an antibody. An enzyme would also be saturated in the case of drug overdose. A remarkable difference is that an enzyme can be used repeatedly and, thus, one enzyme molecule can degrade multiple drug molecules, which is dependent on the turnover number (catalytic rate constant, denoted by k_{cat}). For example, with $k_{\text{cat}} =$ approximately 3000 min^{-1} , one enzyme molecule can degrade up to 3000 drug molecules per minute [19]. Thus, when using an enzyme with a sufficiently large k_{cat} value and high catalytic efficiency ($k_{\text{cat}}/K_{\text{M}}$), all of the drug molecules can be degraded within a short period of time.

The above discussion indicates that enzyme therapy with a sufficiently efficient, drug-specific enzyme would be an ideal approach for therapeutic treatment of acute drug toxicity in the case of drug overdose. It has been extremely challenging to design an appropriate enzyme with a high catalytic efficiency specific for a given drug of abuse, but our laboratory has recently developed novel computational design strategies and protocols for rational design of high-activity enzymes against a given substrate through virtual screening of transition states [20–26]. By employing the computational-design strategies and protocols developed, promising cocaine-metabolizing enzymes have been designed and discovered, as discussed below.

In addition to the treatment of drug overdose, a drug-metabolizing enzyme is expected to be very valuable for the treatment of drug addiction in the future. Effective enzyme therapy for drug addiction would require the enzyme to have a sufficiently high catalytic efficiency (for a given dose of enzyme); high enough to completely suppress the drug reward in the brain. To completely suppress the drug reward in the brain, the elimination rate of the drug by an

enzyme in plasma must be so fast that the drug concentration in the brain can never reach a 'threshold' value, which may vary with specific drugs. Here, the so-called 'threshold' value of a drug is defined as the minimum drug concentration (in the CNS) capable of generating reward effects of the drug. For treatment of drug addiction, a drug-metabolizing enzyme should not only have a sufficiently high catalytic efficiency, but also an appropriately long circulation time in the body. For a therapeutic enzyme with a given catalytic efficiency against a drug, the longer the circulation time of the enzyme in the body, the more efficient the drug-addiction treatment using the enzyme will be. In addition, the higher the catalytic activity of the enzyme against the drug, the lower the dose required to achieve a certain efficacy for antidrug medication will be. With a given dose of the exogenous enzyme, for example, an enzyme with a twofold improved catalytic efficiency means that the desirable minimum overall catalytic activity may last twofold longer in the body.

Encouraging progress has been made in development of therapeutic enzymes for the treatment of cocaine overdose and addiction. It has been known that the primary cocaine-metabolizing pathway in primates is butyrylcholinesterase (BChE)-catalyzed hydrolysis at the cocaine benzoyl ester [27–30]. Enhancing cocaine metabolism by administration of human BChE [31] or bacterial cocaine esterase (CocE) [32,33] has been recognized as a promising treatment strategy for cocaine overdose and abuse [1,34–36]. Both BChE and CocE have their own advantages and disadvantages. In regards to the advantages of using human BChE, first of all, BChE from human source can be tolerated perfectly in human body. In addition, BChE is very stable under physiological conditions and, thus, BChE has a relatively longer half-life in human body. The disadvantages of using BChE are associated with the low catalytic efficiency of native BChE against the naturally occurring (–)-cocaine ($k_{\text{cat}} = 4.1 \text{ min}^{-1}$ and $K_{\text{M}} = 4.5 \text{ }\mu\text{M}$) [37]. Compared with native BChE, the catalytic efficiency of native CocE against (–)-cocaine is approximately 800-fold higher and CocE can be produced on a large scale with relatively low costs. Disadvantage for the use of native CocE and its bacterial origin and low thermostability, with a rather short half-life *in vivo* (~10 min). Hence, the two types of potential treatment agents, BChE and CocE, can complement each other. Both BChE and CocE could be engineered to become valuable anticocaine therapeutic agents. The use of CocE as an anticocaine therapeutic requires a decrease in the immunogenicity and an increase in the thermostability of the enzyme, whereas the use of an engineered BChE as anticocaine therapeutic requires an improved catalytic efficiency against (–)-cocaine.

Through integrated computational experimental studies, we have successfully designed and discovered various high-activity mutants of human BChE [20,22,24–26,38], including the A199S/F227A/S287G/A328W/Y332G mutant ($k_{\text{cat}} = 5700 \text{ min}^{-1}$ and $K_{\text{M}} = 3.1 \text{ }\mu\text{M}$) with an approximately 2000-fold improved catalytic efficiency ($k_{\text{cat}}/K_{\text{M}}$) against (–)-cocaine compared with wild-type BChE [22]. With $k_{\text{cat}} = 5700 \text{ min}^{-1}$, each enzyme molecule of this BChE mutant can hydrolyze up to 5700 (–)-cocaine molecules per minute. Based on the promising *in vitro* activity data, further *in vivo* studies on mice and rats revealed that these high-activity mutants of human BChE protected the animals from the acute toxicity of a lethal dose (180 mg/kg for mice [22,38] or 100 mg/kg for rats [39]) of cocaine and provided rescue of rats after convulsions commenced [39]. In addition, *in vivo* studies on rats [39],

demonstrated that our previously reported A199S/S287G/A328W/ Y332G mutant [20] fused with human serum albumin selectively blocked cocaine-induced reinstatement of drug seeking in rats that had previously self-administered cocaine.

In addition, thermostable mutants of CocE have been designed and discovered [40–45]. The CocE mutants demonstrated a much longer half-life at physiological temperature (37°C), ranging from hours to days [40,41]. The thermo stable mutants of CocE can indeed protect animals against the toxic effects of (–)-cocaine for a much longer period of time [40–43]. Further *in vivo* studies demonstrated the prevention and reversal of cocaine-induced cardiovascular effects in rats by CocE [44], and that polyethyleneglycolylated CocE has been developed successfully for protection against protease digestion and immunogenicity [45].

The general concept of enzyme therapy development for the treatment of cocaine overdose and addiction may also be used to explore possible enzymes suitable for the treatment of other drugs of abuse. In order to explore a therapeutically useful enzyme for a given drug of abuse, one will first need to examine all possible metabolic pathways of the drug and identify a favorable metabolic pathway producing biologically inactive metabolites. If a favorable metabolic pathway and the corresponding native enzyme can be identified, then the aforementioned general computational-design approaches may be used to design high-activity mutants of the chosen drug-metabolizing enzyme against the drug. When necessary, further computational design will be performed to extend the *in vivo* half-life of the discovered enzyme so that the enzyme can be long-acting.

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Bibliography

1. Gorelick DA. Pharmacokinetic approaches to treatment of drug addiction. *Expert Rev Clin Pharmacol*. 2008; 1:277–290. [PubMed: 24422652]
2. Moreno AY, Janda HD. Immunopharmacotherapy: vaccination strategies as a treatment for drug abuse and dependence. *Pharmacol Biochem Behav*. 2009; 92:199–205. [PubMed: 19350728]
3. Redish AD. Addiction as a computational process gone awry. *Science*. 2004; 306:1944–1947. [PubMed: 15591205]
4. Novak G, Seeman P, Le Foll B. Exposure to nicotine produces an increase in dopamine D2(high) receptors: a possible mechanism for dopamine hypersensitivity. *Int J Neurosci*. 2010; 120:691–697. [PubMed: 20942582]
5. Novak M, Halbout B, O'Connor EC, et al. Incentive learning underlying cocaine-seeking requires mGluR5 receptors located on dopamine D1 receptor-expressing neurons. *J Neurosci*. 2010; 30:11973–11982. [PubMed: 20826661]

6. Flatscher-Bader T, Wilce PA. The effect of alcohol and nicotine abuse on gene expression in the brain. *Nutr Res Rev.* 2009; 22:148–162. [PubMed: 19900347]
7. Koob GF, Volkow ND. Neurocircuitry of addiction. *Neuropsychopharmacology.* 2010; 35:217–238. [PubMed: 19710631]
8. Bhattacharyya S, Crippa JA, MartinSantos R, Winton-Brown T, Fusar-Poli P. Imaging the neural effects of cannabinoids: current status and future opportunities for psychopharmacology. *Curr Pharm Des.* 2009; 15:2603–2614. [PubMed: 19689331]
9. Huang X, Zhan CG. How dopamine transporter interacts with dopamine: insights from molecular modeling and simulation. *Biophys J.* 2007; 93:3627–3639. [PubMed: 17704152]
10. Beuming T, Kniazeff J, Bergmann ML, et al. The binding sites for cocaine and dopamine in the dopamine transporter overlap. *Nat Neurosci.* 2008; 11:780–789. [PubMed: 18568020]
11. Huang X, Gu H, Zhan CG. Mechanism for cocaine blocking the transport of dopamine: insights from molecular modeling and dynamics simulations. *J Phys Chem B.* 2009; 113:15057–15066. [PubMed: 19831380]
12. Kinsey BM, Kosten TR, Orson FM. Anti-cocaine vaccine development. *Expert Rev Vaccines.* 2010; 9:1109–1114. [PubMed: 20822352]
13. Leader AE, Lerman C, Cappella JN. Nicotine vaccines: will smokers take a shot at quitting? *Nicotine Tob Res.* 2010; 12:390–397. [PubMed: 20185516]
14. Moreno AY, Azar MR, Warren NA, Dickerson TJ, Koob GF, Janda KD. A critical evaluation of a nicotine vaccine within a self-administration behavioral model. *Mol Pharmacol.* 2010; 7:431–441.
15. Norman AB, Tabet MR, Norman MK, Tsibulsky VL. Using the self-administration of apomorphine and cocaine to measure the pharmacodynamic potencies and pharmacokinetics of competitive dopamine receptor antagonists. *J Neurosci Methods.* 2010 (Epub ahead of print). 10.1016/j.jneumeth.2010.10.017
16. Hubbard JJ, Laurenzana EM, Williams DK, Gentry WB, Owens SM. The fate and function of therapeutic anti-addiction monoclonal antibodies across the reproductive cycle of rats. *J Pharmacol Exp Ther.* 2010 (Epub ahead of print). 10.1124/jpet.110.175083
17. Zhan CG, Zheng F, Landry DW. Fundamental reaction mechanism for cocaine hydrolysis in human butyrylcholinesterase. *J Am Chem Soc.* 2003; 125:2462–2474. [PubMed: 12603134]
18. Landry DW, Zhao K, Yang GXQ, Glickman M, Georgiadis TM. Antibody catalyzed degradation of cocaine. *Science.* 1993; 259:1899–1901. [PubMed: 8456315]
19. Yang W, Xue L, Fang L, Zhan CG. Characterization of a high-activity mutant of human butyrylcholinesterase against (–)-cocaine. *Chem Biol Interact.* 2010; 187:148–152. [PubMed: 20060817]
20. Pan Y, Gao D, Yang W, et al. Computational redesign of human butyrylcholinesterase for anticocaine medication. *Proc Natl Acad Sci USA.* 2005; 102:16656–16661. [PubMed: 16275916]
21. Pan Y, Gao D, Yang W, Cho H, Zhan CG. Free energy perturbation (FEP) simulation on the transition-states of cocaine hydrolysis catalyzed by human butyrylcholinesterase and its mutants. *J Am Chem Soc.* 2007; 129:13537–13543. [PubMed: 17927177]
22. Zheng F, Yang W, Ko MC, et al. Most efficient cocaine hydrolase designed by virtual screening of transition States. *J Am Chem Soc.* 2008; 130:12148–12155. [PubMed: 18710224]
23. Pan Y, Gao D, Zhan CG. Modeling the catalysis of anti-cocaine catalytic antibody: competing reaction pathways and free energy barriers. *J Am Chem Soc.* 2008; 130:5140–5149. [PubMed: 18341277]
24. Yang W, Pan Y, Zheng F, Cho H, Tai HH, Zhan CG. Free energy perturbation (FEP) simulation on transition states and design of high-activity mutants of human butyrylcholinesterase for accelerating cocaine metabolism. *Biophysical J.* 2009; 96:1931–1938.
25. Yang W, Pan Y, Fang L, Gao D, Zheng F, Zhan CG. Free-energy perturbation simulation on transition states and highactivity mutants of human butyrylcholinesterase for (–)-cocaine hydrolysis. *J Phys Chem B.* 2010; 114:10889–10896. [PubMed: 20677742]
26. Zheng F, Yang W, Xue L, Hou S, Liu J, Zhan CG. Design of high-activity mutants of human butyrylcholinesterase against (–)-cocaine: structural and energetic factors affecting the catalytic efficiency. *Biochemistry.* 2010; 49:9113–9119. [PubMed: 20886866]

27. Gao D, Zhan CG. Modeling effects of oxyanion hole on the ester hydrolyses catalyzed by human cholinesterases. *J Phys Chem B*. 2005; 109:23070–23076. [PubMed: 16854005]
28. Gao D, Cho H, Yang W, et al. Computational design of a human butyrylcholinesterase mutant for accelerating cocaine hydrolysis based on the transition-state simulation. *Angew Chem Int Ed*. 2006; 45:653–657.
29. Zhan CG, Gao D. Catalytic mechanism and energy barriers for butyrylcholinesterase-catalyzed hydrolysis of cocaine. *Biophys J*. 2005; 89:3863–3872. [PubMed: 16319079]
30. Hamza A, Cho H, Tai HH, Zhan CG. Molecular dynamics simulation of cocaine binding with human butyrylcholinesterase and its mutants. *J Phys Chem B*. 2005; 109:4776–4782. [PubMed: 16851561]
31. Zhan, CG. Modeling reaction mechanism of cocaine hydrolysis and rational drug design for therapeutic treatment of cocaine abuse. In: Gupta, SP., editor. *Topics in Heterocyclic Drugs* (Volume 4). Springer-Verlag; Heidelberg, Germany: 2006. p. 107-159.
32. Cooper ZD, Narasimhan D, Sunahara RK, et al. Rapid and robust protection against cocaine-induced lethality in rats by the bacterial cocaine esterase. *Mol Pharmacol*. 2006; 70:1885–1891. [PubMed: 16968810]
33. Ko MC, Bowen LD, Narasimhan D, et al. Cocaine esterase: interactions with cocaine and immune responses in mice. *J Pharmacol Exp Ther*. 2007; 320:926–933. [PubMed: 17114567]
34. Zheng F, Zhan CG. Recent progress in protein drug design and discovery with a focus on novel approaches to the development of anti-cocaine medications. *Future Med Chem*. 2009; 1:515–528. [PubMed: 20161378]
35. Zheng F, Zhan CG. Structure-and-mechanism-based design and discovery of therapeutics for cocaine overdose. *Org Biomol Chem*. 2008; 6:836–843. [PubMed: 18292872]
36. Zheng F, Zhan CG. Rational design of an enzyme mutant for anti-cocaine therapeutics. *J Comput Aided Mol Des*. 2008; 22:661–671. [PubMed: 17989928]
37. Sun H, Pang YP, Lockridge O, Brimijoin S. Re-engineering butyrylcholinesterase as a cocaine hydrolase. *Mol Pharmacol*. 2002; 62:220–224. [PubMed: 12130672]
38. Xue L, Ko MC, Tong M. Design, preparation and characterization of high-activity mutants of human butyrylcholinesterase specific for detoxification of cocaine. *Mol Pharmacol*. 2010 (Epub ahead of print). 10.1124/mol.110.068494
39. Brimijoin S, Gao Y, Anker JJ. A cocaine hydrolase engineered from human butyrylcholinesterase selectively blocks cocaine toxicity and reinstatement of drug seeking in rats. *Neuropsychopharmacology*. 2008; 33:2715–2725. [PubMed: 18199998]
40. Gao D, Narasimhan DL, Macdonald J, et al. Thermostable variants of cocaine esterase for long-time protection against cocaine toxicity. *Mol Pharmacol*. 2009; 75:318–323. [PubMed: 18987161]
41. Brim RL, Nance MR, Youngstrom DW. A thermally stable form of bacterial cocaine esterase: a potential therapeutic agent for treatment of cocaine abuse. *Mol Pharmacol*. 2010; 77:593–600. [PubMed: 20086035]
42. Collins GT, Brim RL, Narasimhan D, et al. Cocaine esterase prevents cocaine-induced toxicity and the ongoing intravenous self-administration of cocaine in rats. *J Pharm Exp Ther*. 2009; 331:445–455.
43. Narasimhan D, Nance MR, Gao D, et al. Structural analysis of thermostabilizing mutations of cocaine esterase. *Protein Eng Des Sel*. 2010; 23:537–547. [PubMed: 20436035]
44. Wood SK, Narasimhan D, Cooper Z, et al. Prevention and reversal by cocaine esterase of cocaine-induced cardiovascular effects in rats. *Drug Alcohol Depend*. 2010; 106:219–229. [PubMed: 19800183]
45. Park JB, Kwon YM, Lee TY, et al. PEGylation of bacterial cocaine esterase for protection against protease digestion and immunogenicity. *J Control Release*. 2010; 2142:174–179. [PubMed: 19857534]

Websites

101. UNODC. World Drug Report 2010. United Nations Publication; 2010. Sales No. E.10.XI.13 www.unodc.org/documents/wdr/WDR_2010/World_Drug_Report_2010_lores.pdf

102. National Institute on Drug Abuse (NIDA) info facts. www.drugabuse.gov/infofacts/understand.html