Recent Advances for the Treatment of Cocaine Abuse: Central Nervous System Immunopharmacotherapy

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ABSTRACT

Cocaine addiction continues to be a major health and societal problem in spite of governmental efforts devoted toward educating the public of the dangers of illicit drug use. A variety of pharmacotherapies and psychosocial programs have been proposed in an effort to provide a method for alleviation of the physical and psychological symptoms of cocaine abuse. Unfortunately, these methods have been met with limited success, illustrating a critical need for new effective approaches for the treatment of cocaine addiction. Recently an alternative cocaine abuse treatment strategy was proposed using intranasal administration of an engineered filamentous bacteriophage displaying cocaine-sequestering antibodies on its surface. These phage particles are an effective vector for CNS penetration and are capable of binding cocaine, thereby blocking its behavioral effects in a rodent model. The convergence of phage display and immunopharmacotherapy has allowed for an investigation of the efficacy of protein-based therapeutics acting within the CNS on the effects of cocaine in animal models and has uncovered a new tool in the battle against cocaine addiction.

KEYWORDS: cocaine, central nervous system, immunopharmacotherapy, virus, phage display

THE PROBLEM OF COCAINE ADDICTION

The addiction syndrome is remarkably similar across different drug classes, primarily characterized by a chronic relapsing brain disorder with associated neurobiological changes that lead to a compulsion to take a drug without regard for drug intake.^{1,2} The dopamine hypothesis of reward, a common pathway of addiction, has recently been evolving, with the mesocorticolimbic dopaminergic system now viewed as central to both natural rewards and drug-seeking behavior,

though perhaps having a more minor role in the maintenance of such behavior.³⁻⁵ Cocaine, a powerful psychostimulant, triggers mechanisms in the brain as fundamental as those activated by food, water, and sexual activity. In essence, the effects of drug consumption are euphoric and stimulating, while the absence of the drug leads to dysphoria and depression. However, this does oversimplify the phenomenon as the transition from drug use to drug abuse is exceedingly complex since both positive and negative reinforcement phenomena are involved. Nonetheless, it is surprising that the most sought and abused drugs of abuse comprise a small set of naturally occurring molecules and closely related derivatives; among these compounds are such drugs as cocaine, nicotine, amphetamines, and opiates.

Cocaine (Figure 1) is a tropane alkaloid extracted from the leaves of the native South American plant *Erythroxylon coca*. Although the most prevalent instances of the problems associated with cocaine have emerged in modern civilization, human cocaine use has likely occurred for thousands of years.⁶ Cocaine is purified from coca leaves (containing between 0.6% and 1.8% alkaloidal cocaine) using a relatively simple method by which it is extracted from the leaves with an organic solvent (often kerosene), resulting in a coca paste containing approximately 80% cocaine. The alkaloids are passed through an acidic aqueous solution based on hydrochloric acid; the solution is neutralized and the cocaine is extracted by recrystallization. Cocaine hydrochloride (HCl) is the pharmaceutical form used as a local anesthetic, and abused by drug addicts. It is vulnerable to pyrolysis, resulting in a poor rewarding effect when smoked. However, upon transformation into cocaine freebase (popularly termed "crack" or "rock" cocaine) by dissolving it in an alkaline solution followed by precipitation, the hydrochloride salt transforms into a smokable, pyrolysis-resistant material. Most frequently, cocaine is used in the form of a powder. It can be introduced into the body by sniffing, swallowing, or injecting to produce its characteristic effects as cocaine is readily absorbed by all mucous membranes, such as the lining of the mouth, nasal passages, and gastrointestinal tract.

The abuse of cocaine is maintained by the drug's effects on brain reward systems, and mediated at least in part by its dopaminergic action. The patterns and consequences of use

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Figure 1. Structure of cocaine and its nonpsychoactive metabolite methyl ecgonine. Antibodies capable of catalyzing this reaction have been a topic of significant interest for various research groups in an effort to design biocatalysts for the treatment of cocaine addiction.

are best understood by considering the pharmacokinetics (rapid absorption and delivery to the brain, relatively short half-life), the pharmacodynamics (intense central and peripheral neural stimulation), and the route of drug administration. Cocaine is used therapeutically as a topical and local anesthetic. Toxicity occurs primarily in cocaine abusers, but also occasionally after therapeutic dosing. Medical complications reflect primarily excessive central nervous system (CNS) stimulation and excessive vasoconstriction, the latter resulting in severe hypertension and/or organ ischemia with associated organ injury. Most deaths that result from medical complications of cocaine intoxication are sudden and occur before medical intervention is possible. Other complications of cocaine abuse with severe personal and social consequences include traumatic deaths and injuries, reproductive disturbances, and transmission of infectious diseases, especially AIDS. Recent surveys for cocaine abuse in the United States have indicated that more than 23 million people have tried cocaine, nearly 400 000 use it daily, and 5000 new users are added each day, despite the listing of this compound by the Drug Enforcement Agency as a Schedule II agent.⁷ Although abuse appears to be stabilizing relative to the more rampant use of cocaine observed in the 1980s, as much as 0.3% of the population may be dependent on the drug.⁸ A myriad of medical problems, including death, often accompany cocaine use and the association of the drug with the spread of AIDS is of great concern.^{9,10} Furthermore, the detrimental effects are especially tragic for pregnant women where “crack” has been reported the most abused illicit drug.¹¹ From the behavioral pharmacology, it has been evident that cocaine is highly addictive and may be the most reinforcing of all commonly abused drugs.¹²

COCAINE PHARMACOTHERAPY

Cocaine addiction represents a serious social and health problem, which has led the National Institute on Drug Abuse (NIDA) to urge the scientific research community for the development of an effective pharmacological treatment for

this condition. However, pharmacotherapies of cocaine addiction that typically target the monoaminergic neurochemical substrates implicated in its reward properties, have as yet been unsuccessful, and often generate adverse side effects.

Extensive research efforts have been devoted to the development of an effective pharmacotherapy for the treatment of cocaine abuse. However, unlike the historically successful methadone treatment for heroin addiction, there is no proven pharmacotherapy for cocaine abuse.¹³ In recent years, more than 2 dozen medications have been tested as potential therapeutic agents in the treatment of cocaine dependence.¹⁴ A significant component of this effort is to rapidly screen currently marketed drugs to identify potential lead compounds for further testing. Of the compounds tested, 4 (cabergoline,¹⁵ reserpine,¹⁶ sertraline,¹⁷ and tiagabine¹⁸) have been advanced to further phase II clinical trials, and 2 others (disulfiram¹⁹ and selegiline²⁰) have proceeded or are soon expected to proceed to phase III trials. Other compounds, including baclofen,²¹ topiramate,²² and modafinil²³ have also advanced into midstage clinical development. The approaches underlying these compounds can be divided into 3 key areas¹⁵: (1) Those compounds that can be used in a substitution-based treatment as a cross-tolerant stimulant; (2) medications that serve as antagonists by blocking the binding of cocaine to its cognate receptors; and (3) compounds that function by acting at other sites distinct from the cocaine site of action but functionally antagonize the effects of cocaine. Several biopsychosocial models have been proposed and evaluated to address addiction and relapse prevention.²⁴ Unquestionably, an improved pharmacotherapy would increase the effectiveness of such programs and alternative strategies for treating cocaine addiction are needed if progress is to be made.

The use of protein-based therapeutics has been employed as a strategy for the treatment of cocaine addiction. In this approach, proteins are designed to bind cocaine, thereby blocking its effects and/or degrading the drug, rendering it less psychoactive.²⁵ Over the past decade, our group and later additional laboratories have reported the successful blocking of the psychostimulant effects of cocaine by anticocaine antibodies with both active and passive immunization in rodent models. These results demonstrate that anticocaine antibodies bind to cocaine in circulation, retarding its ability to enter the brain.²⁶⁻³⁰ Additionally, behavioral studies into cocaine-induced locomotor activity and self-administration have revealed that both strategies have some efficacy in rats. A related antibody-based approach to cocaine addiction treatment used catalytic antibodies specific for cocaine and the cleavage of its benzoyl ester (Figure 1).³¹⁻³⁶ The efficiency of catalytic antibodies has been demonstrated in rodent models of cocaine overdose and reinforcement, but kinetic constants for all reported antibody catalysts are marginal and thus improved rates

will be required before clinical development is warranted.³⁷ The development of cocaine vaccines using both active and passive immunization strategies and the advancement of these vaccines into clinical trials has been recently reviewed.^{37,38} Finally, groups using butyrylcholinesterase (BChE),^{39,40} the major cocaine-metabolizing enzyme present in the plasma of humans and other mammals, have reported that intravenous pretreatment with either wild-type or genetically engineered BChE can mitigate the behavioral and physiological effects of cocaine and accelerate its metabolism.⁴¹⁻⁴³ The main drawback common to all of these protein-based approaches is that none acts directly within the CNS; thus, their success depends solely on peripheral contact between the enzyme or antibody with ingested cocaine.

BACTERIOPHAGE AND ITS THERAPEUTIC APPLICATION

Bacteriophage are viruses that infect bacteria, and are distinct from the animal and plant viruses in that they lack intrinsic tropism for eukaryotic cells.⁴³ There are an estimated $>10^{30}$ phages in the biosphere and since phage particles typically outnumber prokaryotic cells by approximately 10-fold in environmental samples, phages probably constitute an absolute majority of organisms on our planet. In particular, filamentous bacteriophage can be produced at high titer in bacterial culture, making production simple and economical. Additionally, they are extremely stable under a variety of harsh conditions, including extremes in pH and treatment with nucleases or proteolytic enzymes.⁴⁴ But perhaps the most significant advantage is the genetic flexibility of filamentous phage. This property was exemplified in the seminal paper of Smith in which a method was reported that physically linked genotype and phenotype in a protein display system, now known as phage display.⁴⁵ In this work, foreign cDNA was fused to the gene encoding the surface protein pIII; thus, the foreign cDNA was transcribed and translated as a fusion protein with pIII and displayed on the phage surface. This enabled the researcher to actually select

displayed proteins for specific properties and to recover the gene encoding this protein.

The filamentous phage M13 is approximately 895 nm long and 9 nm in diameter. Its single-stranded DNA genome contains 6407 bases that encode 10 different proteins. The DNA is enclosed in a protein coat composed of approximately 2800 copies of the gene VIII protein (pVIII). A viable phage also expresses 3 to 5 copies of pIII on its tip. Unlike most other bacteriophage, M13 does not produce a lytic infection in *Escherichia coli*, but rather induces a state in which infected host cells produce and secrete phage particles without undergoing lysis. Using this powerful technology, a wide variety of proteins, antibodies, and peptides can be displayed on the phage coat (Figure 2).

Over the past 2 decades, many important developments in the field of phage display have occurred. Very large libraries of proteins of assorted sizes, ranging from small peptides and antibody fragments to functional enzymes, can now be displayed on various coat proteins of the phage particle, allowing for the identification of natural or artificial ligands for a wide range of receptors, the selection of antibodies with high specificity and affinity against a given antigen, the targeted delivery of foreign DNA into cells, and the selection of enzymes with improved functionality, just to name a few possibilities of phage display technology.

The use of bacteriophage as a therapeutic agent has been known since the 1920s, when lytic phage were administered as antibacterial agents.⁴⁶ Their further application was hampered by several factors, of which the inability to remove endotoxins from preparations was predominant. More recently, animal studies have demonstrated that bacteriophage can rescue animals from a variety of fatal infections,⁴⁷ while studies conducted in Eastern Europe have shown that phage can be effective in treating drug-resistant infections in humans.^{48,49} These encouraging findings have resulted in the emergence of several biotechnology companies embracing the use of phage technology as a therapeutic option, and the US Food and Drug Administration may soon issue regulatory guidelines for phage therapy.

Figure 2. Filamentous phage *fd* architecture. The phage coat contains 2800 copies of the major coat protein pVIII, whereas there are 5 molecules of each of the minor coat proteins pIII, pVII, and pIX.

The use of filamentous phage in vivo has been previously reported by Pasqualini and Ruoslahti.⁵⁰ In this work, bacteriophage displaying a random peptide library was intravenously injected into mice and subsequently rescued from the internal organs, showing that the integrity of the phage was not compromised.⁵¹ The ability of bacteriophage to penetrate a wide range of vertebrate tissues without detrimentally affecting the host has also been recently reviewed.⁵² Furthermore, the ability of filamentous phage to penetrate the CNS has also been reported.⁵³ Here, Frenkel and Solomon were able to deliver phage-displayed anti- β -amyloid antibodies via intranasal administration into the brains of mice. Frenkel and Solomon's report was significant as it provided the following findings: (1) filamentous phage can easily access the CNS; (2) phage can display foreign proteins on its surface and still penetrate the CNS; (3) bacteriophage can be injected intranasally multiple times into the same animals without visible toxic effects as evidenced by brain histology; and (4) the behavior and life span of all treated animals was monitored for 1 year and no harmful effects were found.

Previously, we have shown that sequestration of cocaine by anticocaine antibodies can suppress the psychomotor and reinforcing actions of the drug.²⁶⁻²⁸ From these studies, a murine monoclonal antibody termed GNC92H2 emerged that has exquisite affinity and specificity to cocaine ($K_d = 40$ nM; while for its hydrolysis product benzoyl ecgonine $K_d = 1.4$ μ M).²⁶ Within our laboratories, we have also demonstrated that antibody libraries can be displayed on the filamentous phage coat proteins pIII, pVII, and pIX for the selection of high-affinity antibodies to a wide variety of antigens.^{54,55} Combining the results of Frenkel and Solomon with our previous studies in cocaine immunopharmacotherapy, we hypothesized that antibodies or enzymes specific for cocaine that are displayed on filamentous phage will have an unprecedented ability to access the CNS. Consequently, this enables for the first time, an investigation of how protein-based therapeutics acting in the CNS can influence the effects of cocaine in rat models.⁵⁶ In contrast to more traditional methods such as active or passive immunization, intranasal administration of bacteriophage-derived protein therapeutics provides a direct route for entry into the CNS. The advantage to this method stems from the fact that although antibodies may bind cocaine effectively, these biomacromolecules are unable to quantitatively remove cocaine from the bloodstream prior to CNS entry. Thus, the presence of a bacteriophage-bound cocaine-binding molecule within the CNS would allow for enhanced removal of the drug from the user's system.

TREATING COCAINE ADDICTION WITH VIRUSES

To achieve a greater number of anticocaine antibodies on the phage surface, we chose the major coat protein pVIII as

fusion partner for the scFv antibody molecule (Figure 3), thus hoping to generate a "molecular sponge" to absorb cocaine. The affinity of GNC 92H2-pVIII for cocaine was determined by equilibrium dialysis to be between 50 nM and 5 μ M, depending on the number of scFv 92H2 antibodies displayed on each phage particle. Thus, we surmised that the phage-displayed scFv 92H2 construct possessed comparable cocaine affinity to free scFv in solution.

To assess the efficacy of immunization with phage display antibodies within the CNS the psychostimulant effects of cocaine were measured in the rat. This psychostimulant effect is a dose-dependant increase in locomotor activity and stereotyped behavior as a result of cocaine's actions on dopaminergic neurons in the brain. Three different doses of cocaine were chosen for study: 10, 15, and 30 mg/kg. These doses of cocaine represent a broad range of both locomotor and behavioral responses. Thus, the lowest dose produces little locomotor and virtually no stereotyped behavior, whereas the medium dose produces a significant locomotor activation and modest stereotyped behavior and the highest dose produces less locomotor activity but more robust stereotyped behavior.

Animals were treated twice per day by intranasal administration for 3 consecutive days with phage displaying single chain antibodies on their pVIII surfaces. In these experiments, the antibodies used included GNC 92H2-pVIII and RCA₆₀28-pVIII, a control antibody that has excellent affinity (400 nM) and selectivity to RCA₆₀ (*Ricinus communis* agglutinin, "ricin").⁵⁴ In comparison to gene pIII,

Figure 3. Enlarged region of the phage coat showing coat protein pVIII displaying a single-chain antibody (scFv).

which can only exhibit up to 5 copies on the surface of a phage, the pVIII gene was chosen as it contains 2800 copies and was anticipated to provide an overall higher concentration of protein on the phage surface.⁵⁷ Protein surface concentration is a key element in success of this approach as the antibodies displayed were not catalytic; hence, sequestering was the only means of inhibiting cocaine from reaching its target. Monoclonal antibody GNC 92H2 has previously been shown to have excellent avidity and specificity to cocaine and has yielded outstanding results in previous passive immunization behavioral studies.²⁶⁻²⁸

Over 4 consecutive days, the immunized animals received daily intraperitoneal (IP) cocaine challenges of 1 of 3 doses of the drug, 4 days after the onset of the phage-infusion regime. Intranasal administration of phage GNC 92H2-pVIII versus RCA₆₀28-pVIII resulted in significant psychomotor differences between groups in response to cocaine

(Figure 4). At the 10 mg/kg dose, a 30% reduction in ambulatory behavior (crossovers) compared with baseline values was observed in the GNC 92H2-pVIII group but not in controls and this difference was reflected in the stereotypy measurement during the first 10 minutes of the session. This modest difference in ambulation is not surprising given the tenuous hypermotility elicited at this dose. Furthermore, the paucity of the observed stereotypy is consistent with previous reports using a low dose of cocaine.⁵⁷ In contrast, a marked 47% decrease in locomotor activity was measured in GNC 92H2-pVIII-treated animals versus baseline values, while controls increased their overall responses by 11%. This quantitative trend was also observed in the percent stereotypy displayed, where the behavior was rated in controls up to 70 minutes into the session, as opposed to 50 minutes in the GNC 92H2-pVIII group. These results have a striking similarity to our previously reported results using both active immunization with 2 different cocaine conjugates²⁶⁻²⁸ and passive immunization with the mAb GNC 92H2.²⁸ This

Figure 4. Locomotor activity (crossovers; *Upper*) and stereotyped behavior (sniffing and rearing; *Lower*) after IP injection of cocaine after nasal immunization with GNC 92H2-pVIII (●) or RCA₆₀28-pVIII (□). The figure shows the response to postnasal immunization cocaine challenge at 10 (a), 15 (b), and 30 (c) mg/kg. *Upper* values represent means $\pm$ SEM of 16 animals ($n = 8$). * $P < .05$ ANOVA, significant difference between groups. *Lower* data represent the percentage of incidence of the observed behavior. * $P < .05$.

similarity probably is contingent upon 2 main experimental factors. First, the same cocaine dose was used, therefore, the patterns of hyperactivity are congruent. Second, the cocaine-blocking mechanism, albeit central versus peripheral, still obeys an immune-mediated dynamic, which is subject to the same elements of affinity and titer surmountability. This so-called element of surmountability is most evident in the data depicted by Figure 4C. At the 30-mg/kg cocaine dose, a reversal of behavioral profile was obtained, whereby control animals showed diminishing levels of locomotion compared with both their own baseline values and GNC 92H2-pVIII-treated rats during the first 30 minutes of the session (Figure 4C). Interestingly, stereotyped behavior was sustained by controls significantly longer and at higher percentages than by the GNC 92H2-pVIII group (Figure 4C) reflecting the typical emergence of increased levels of this measure at the higher doses of cocaine.⁵⁸ Therefore, the apparent absence of a blunting effect in locomotor activity in the GNC 92H2-pVIII-treated animals versus controls may instead be interpreted as an absence of group differences by virtue of a decrease in ambulation by control animals as their repetitive (stereotypic) behavior increased and endured.

Confirmation of the basis of the behavioral suppression was next studied by measuring the phage titer in the brain before, during, and after the time span of animal behavioral studies. The earliest time point at which phage were observed in the brain was at day 2, while the highest titer of phage observed in the brain was at day 4. Although high phage titers dropped precipitously from day 5 to day 7 (10^3), this level was maintained until day 15 and was not subsequently undetectable by day 17. Thus, upon subsequent challenges, (ie, day 4 and beyond), there were no significant differences in either motor measure between groups.

In understanding the role of any nasal vaccine, we felt it was important to investigate potential limitations. The CNS is considered an immune privileged site, however, the possibility of phage entering the periphery cannot be discounted. Filamentous phage in itself, and with displayed proteins on its surface, comprises a foreign entity to the immune system. Additionally, there is a growing body of research wherein nasal vaccination has become increasingly popular.^{59,60} Gratifyingly, enzyme-linked immunosorbent assay (ELISA) analysis of rat serum from vaccinated animals showed no appreciable antibody titer to phage and thus provided further indication that potential toxic side effects are not being manifested in animals that were administered filamentous phage.^{50,52,53}

CONCLUSIONS

The compilation of these studies has demonstrated a promising new strategy in the continuing effort to develop effective

treatments for cocaine addiction. One of the key strengths of this approach in relation to previous immunopharmacotherapeutic strategies is the direct delivery of the therapeutic protein into the CNS, the primary site of drug action. Thus, convergence of phage display and immunopharmacotherapy has enabled, for the first time, an investigation of how a protein-based therapeutic acting within the CNS can influence the effects of cocaine in animal models. Future investigations in the context of cocaine abuse could include the combination of this phage-based approach with either passive or active immunization protocols to determine whether any synergistic benefits can be obtained from simultaneous peripheral and central immunization. Furthermore, prior to the advancement of this technology into a clinical scenario, a long-term study of intranasal phage administration is essential to assess the effect of chronic phage administration on brain structure and/or function.

As with all other pharmacotherapeutic approaches, a significant concern lies in the ability of a cocaine user to surmount the effects of a given medication by simply administering higher doses of the drug, with potentially lethal consequences. Furthermore, although cocaine-binding antibodies may prevent the effects of cocaine, this treatment does not abrogate the psychological need to take the drug. Clearly, in order to successfully recover from cocaine addiction, any pharmacotherapeutic approach should be performed under controlled conditions under the supervision of a medical professional in conjunction with suitable psychological treatment (eg, counseling). However, encouraging recent data have emerged suggesting that the issue of overcoming a vaccine may prove manageable in humans.³⁸

However, this does not imply that intranasal administration of protein-based therapies for drug abuse treatment is limited to binding antibodies or peptide ligands only. Catalytic antibodies could be effective in cocaine abuse treatment as one equivalent of catalytic antibody can turnover many times; thus less protein would be required for a given pharmacological effect relative to binding antibodies. Furthermore, enzymes such as the bacterial cocaine esterase⁶¹ (CocE) or butyrylcholinesterase (BChE) show great promise in this area. CocE is the most efficient biocatalyst known for cocaine degradation. However, direct administration of this enzyme would result in both extensive proteolysis prior to action and a likely immune response directed against the enzyme. Based on our results, display on phage should attenuate this immune response and allow these proteins to become viable therapeutics. The combination of catalysis and the potentially large protein concentration attainable on the phage surface using gene pVIII could provide a powerful weapon in the treatment of cocaine addiction. Furthermore, we anticipate that other enzymes relevant to the metabolism and degradation of drugs of abuse could also be applied to this system.

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