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Prospects, Promise and Problems on the Road to Effective Vaccines and Related Therapies for Substance Abuse

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EXECUTIVE SUMMARY

This review addresses potential new treatments for stimulant drugs of abuse, especially cocaine. Clinical trials of vaccines against cocaine and nicotine have been completed with the generally encouraging result that subjects showing high titers of anti-drug antibody experience a reduction in drug reward, which may aid in cessation. New vaccine technologies including gene transfer of highly optimized monoclonal antibodies are likely to improve such outcomes further. In the special case of cocaine abuse, a metabolic enzyme is emerging as an alternative or added therapeutic intervention, which would also involve gene transfer. Such approaches still require extensive studies of safety and efficacy, but they may eventually contribute to a robust form of in vivo drug interception that greatly reduces risks of addiction relapse.

Keywords

cocaine abuse; vaccine; adenovirus gene transfer vector; metabolism-based therapies; monoclonal antibody; butyrylcholinesterase; cocaine hydrolase; Anti-drug immunoglobulin; clinical Trial

INTRODUCTION

According to the 2010 National Survey of Drug Use and Health, over 22 million Americans have substance dependence or abuse problems [1]. Many stimulants do not directly target a clearly defined chemoreceptor, but act on transporters to increase synaptic levels of dopamine, norepinephrine, and serotonin in brain reward centers. For this reason, successful treatment options with receptor antagonists have been difficult to identify. One strategy for overcoming these obstacles and reducing risks of relapse after initial rehabilitation is based on intercepting drugs of abuse before they reach brain reward centers. The major therapeutic candidates to implement such an approach are antibodies and catalytically efficient metabolic enzymes, both of which may be considered pharmacokinetic antagonists of their target drug. A number of vaccines have been developed to elicit high-affinity antibodies

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against different stimulants with high liability for abuse. These vaccines and their clinical efficacy have been discussed in several recent reviews [2–8], but the pace of research remains rapid and the subject merits continued attention. A different but related means of drug interception involves accelerating metabolic clearance to the point where it can also affect the levels of drug reaching the brain. This idea arose not long ago, and, for reasons we will discuss, it so far appears applicable only to a single agent of abuse, namely cocaine. This review will consider progress and obstacles on the path to developing and implementing vaccine and metabolism-based therapies optimal for treatment of cocaine abuse. We will also explore the potential for the two interception approaches to reinforce each other in an additive or even synergistic manner.

Antibodies and vaccines against drugs of abuse

In recent years, the therapeutic scope of vaccines has been broadened by the realization that toxic drugs of various kinds can be prevented from reaching their sites of action if the recipient individual has been vaccinated against a closely related chemical structure. When it comes to addictive substances, it has proved feasible to generate substantial titers of high-affinity antibodies against psychoactive small molecule haptens conjugated to appropriate carrier proteins such as keyhole limpet hemocyanin (KLH), cholera toxin (CTB) [9], and tetanus toxoid (TTX) [5].

It is recognized that any drug-hapten conjugate will elicit a strong antibody response to the carrier protein as well as the intended target, but these responses do not interfere with the production of anti-drug immunoglobulins and anti-carrier antibodies are regarded as innocuous, although they can sometimes affect production of the desired anti-hapten antibodies [10]. The nature of the carrier protein is particularly important in determining the ultimate titer of anti-drug antibodies. One new conjugating agent showing great promise in this regard involves capsid proteins from killed adenovirus [11].

Drug hapten-conjugated vaccines have proven able to reduce the physiological and behavioral responses to their target molecules when tested in experimental animals, typically rodents, but also primates. Encouraging results have been obtained with vaccines to cocaine, heroin, nicotine, and methamphetamine, among other widely abused stimulants [12–15]. Thus, several studies have demonstrated reduced total drug levels in plasma or brains of rodents after passive immunization or after a good vaccine response with high titers of specific drug antibody [16–19].

Such vaccines are capable of eliciting antibodies in molar quantities approximately commensurate with the plasma drug levels attained after typical “rewarding doses”. That point raises both a technical and a substantive issue. First, though the classic immunology literature typically deals with antibody titers in terms of thresholds for detection, molar values are more relevant to questions of balance between drug molecules and ligand binding sites on an immunoglobulin. Second, there is the practical question of how many drug binding sites are needed when the objective is to weaken or eliminate drug actions in the central nervous system, recognizing that each antibody has two binding sites and in theory can bind two drug molecules. In the absence of experimental data, a one-to-one ratio between binding sites and drug molecules would seem a reasonable goal. Published studies, however, indicate that drug-binding immunoglobulins with high affinity affect reward-driven behavior noticeably even when, in terms of local concentration, they are somewhat less abundant than the drug itself [20,21].

Since a sudden rise in brain drug concentration is regarded as key to drug reward [22,23], antibody-induced blunting of reward effects is often ascribed to a broadening of the “drug pulse” created by rapid intake (i.e., reduced rate of rise in drug concentration). Indeed,

turbulent flow allows drug to interact with antibody while passing through the vasculature. IgG distributes throughout the extracellular water compartment, at levels somewhat lower than in blood. Since this compartment is several times larger than plasma volume, the total pool of antibody available to adsorb drug can substantially exceed the total drug dose. Under that condition, specific anti-drug IgG can reduce the concentration of free drug and the rate at which drug concentration rises in the brain. Without a sophisticated dynamic model the exact consequences are unpredictable. However, this line of reasoning suggests that such effects can be physiologically important.

Advantages and problems with anti-drug vaccines

The vaccine approach to treating drug abuse has two great advantages. First, the basic technology is well-understood, robust, cost effective, and easy to accomplish; and second, the risks of treatment are relatively minor both in reality and in the public perception. In our view, the chief problems are these: 1) individual responses can be highly variable (for genetic and other reasons); 2) responses in many human subjects do not eliminate drug reward entirely and may not be sufficient therapy in themselves; 3) there is no ready means of insuring that a large proportion of the antibodies generated are optimal for drug binding; 4) sustained therapy requires booster immunizations and, therefore, continued commitment to remaining drug free; 5) any given level of anti-drug antibody can in principle be saturated by a large or repeated drug dose; 6) a subject taking increased doses, especially of cocaine, may accumulate toxic intermediates that damage vulnerable tissues such as liver or heart (recent work in our laboratories to model such effects will be discussed later). Most of these problems are amenable to further advances in vaccine technology, including better adjuvants and hapten conjugates, and perhaps novel ways of generating and delivering immunoglobulins with ideal properties toward the target agent(s). Improvements can be expected on a number of fronts as the basic science develops. Meanwhile, progress is occurring at the clinical level. It can be argued that existing vaccines already tip the balance between cost (in risk and effort) and drug reward. Further improvements could well cause a noticeable reduction of drug-seeking and drug-intake.

Clinical trials

Clinical trials have been successfully completed on vaccines for cocaine [24] and nicotine [25,26]. The results indicate partial therapeutic effects with both target drugs. Such effects are of course related to the antibody titers achieved. Because these tend to vary substantially among individuals, the *overall* compiled results are modest and, in the case of the cocaine vaccine, not statistically significant. A major encouragement from the cocaine vaccine study by Kosten and colleagues was a statistically significantly increased frequency of drug-free urines [9]. This outcome was impressive given that the participants were regular users with no declared motivation to quit. Another striking feature of this study was the apparent failure of participants to compensate for a hypothesized reduction in perceived reward value of cocaine. Thus, close monitoring of the urine samples revealed no pattern of increase in cocaine metabolites, which would have accompanied a rise in drug intake. Studies at Baylor showed that some subjects developed IgM anti-cocaine antibodies in response to prior “recreational exposure”, and they responded significantly less well to the CTB-conjugate cocaine vaccine [27]. The reason for antibody development is not known, but it is possible that reactive cocaine metabolites combined in vivo with host proteins to form hapten-carrier complexes. One lesson is that future trials must be designed explicitly to stratify participants in terms of anticipated levels of anti-drug antibody response. That would allow the data forwarded to the US FDA or international regulatory bodies to meet expectations of success. It also seems appropriate in future trials to exclude subjects with high titers of pre-existing anti-cocaine IgM, which may arise by mechanisms similar to those operating in the Baylor vaccine trial.

Monoclonal antibodies

New ideas and technologies are emerging to enhance the power of anti-drug vaccines or complement them in synergistic ways. In particular, recent work has yielded notable successes in the form of monoclonal IgG antibodies (mAbs) with very high affinities for target agents such as amphetamine, cocaine, and nicotine [15,18,28,29]. Such antibodies are clearly able to affect reward-driven responding in rodents, although not entirely in the fashion one might expect. For example, with a humanized, mouse-derived mAb to cocaine, Norman and colleagues found that a large dose delivered by i.v. injection (120 mg/kg) led a 3-fold increase in "priming threshold" in rats trained for cocaine-self administration [28]. This effect can be viewed as a sign of reduced reward value for the amount of cocaine delivered. In addition, there was a modest dose-dependent *increase* in cocaine consumption rates, which was interpreted as an attempt to attain the same final reward level that was being attained before antibody administration. These findings imply that the mAb was affecting cocaine delivery to brain reward centers, but to a modest and surmountable extent. One might hope for a complete cessation of responding if interception were maximally effective. This goal may actually be achievable with higher doses of the mAb, or one with still greater affinity for the target. At present, however, even a partial effect of this nature could help individuals and benefit communities.

A chief obstacle to implementing mAbs for drug abuse (passive immunotherapy) is the need for large amounts of purified IgG, in addition to the cost and inconvenience of repeated injections, or the vagaries of sustained release preparations, mini-pumps, and other means of sustained delivery. Drug-specific mAbs might well serve to reverse drug overdose rapidly in emergency situations. However, passively administered antibodies can only remain in the system for a short period of time, assuming normal metabolic clearance. Although considerable therapeutic impact could be gained by selection and protein engineering for ideal drug-binding properties and in vivo behavior, these advantages are compromised when long-term therapy requires the patient to make a series of affirmative decisions to continue with a treatment plan that offers no direct relief or reward. One could argue that the basis for successful treatment should be an approach that is truly "vaccine like" in the sense that an initial decision is followed by steps that are inherently long-lasting, with no need for frequent interventions to maintain them. In this respect, conventional vaccination with the best available carriers and adjuvants lies in the border zone. That is to say, such treatments can provide high titers of anti-drug antibodies over periods much longer than the half-life of circulating IgG molecules, but several revaccination or booster injections per year may be required r to sustain the immune response. This state of affairs can work well for individuals with an appropriate level of motivation. Nonetheless, it is a reason to explore other means for long-term delivery of drug-intercepting proteins.

Gene transfer of anti-drug proteins

Gene transfer can deliver a refined and reproducible therapeutic protein with the durability and compliance-friendly aspects of vaccination. After a hiatus of more than 10 years following the unfortunate outcome of an early clinical trial of protein therapy based on gene transfer [30] this approach is re-emerging as an option for a number of chronic conditions, including clotting disorders [31], immunodeficiency [32], inherited blindness [33], and cancer [34]. Substance abuse and addiction may also prove amenable to gene transfer, in this case by delivering "interceptor molecules" that prevent drug access to brain targets.

The characteristics of ideal gene therapy for any non-fatal illness should be, in rough order of importance: 1) acute safety (no serious immediate adverse effects); 2) chronic safety (no immunological disturbance or disruption of oncogenes in cells harboring delivered DNA and producing transgene product); and 3) high therapeutic impact. To aid in recovery from

addiction one also needs to sustain adequate levels of therapeutic protein during the period with maximal risk of relapse (which can last 2 years or more). Since addiction is not immediately life-threatening, these requirements raise the bar for treatment with viral vectors. But to argue the positive case, recognizing the huge burden that drug addiction imposes on individuals and families, an effective therapy with some degree of risk does seem appropriate.

Despite lingering concerns regarding viral gene transfer, its potential for delivery of "ideal" monoclonal antibodies is well recognized [35,36] and has recently been tested in animal studies on viral transduction of an anti-nicotine IgG. Using an AAVrh.10 vector that expressed full-length antibody derived from the Fab fragment of anti-nicotine mAb NIC9D9, Hicks and colleagues [37] were able to shield mouse brain from systemically administered nicotine, reducing tissue drug concentration approximately 7-fold as compared with unprotected mice. This effect was adequate to block nicotine-stimulated increases in blood pressure, heart rate and locomotor activity. Moreover, the antibody persisted undiminished at high titers for 18 weeks. In fact, the levels of anti-nicotine IgG achieved after injections of 10^{11} viral vector gene copies (moderate viral load) rose to 1000 μg/ml, equivalent to a ~150 μM concentration of drug-binding sites. That was far above what could be expected after classical vaccination, and roughly 10 times higher than the nicotine plasma concentration observed in human volunteers immediately after smoking a high-nicotine brand of cigarettes [38]. Therefore, the highly effective action in mice could have been expected, and it may well translate to human application. The same research group achieved similar success with viral delivery of an anti-cocaine antibody, which suppressed cocaine-induced locomotion for months [39].

Immunological approaches through gene transfer along these lines offer several advantages. First, the molecular properties of the therapeutic can be defined, and refined ahead of time. Second, cost in the long term should be far lower, as there is no need for repeated large-scale culture fermentations and purification. Third, a single treatment should suffice to generate therapeutic levels of antibody long enough to stabilize abstinence—i.e., more than a year and perhaps indefinitely. The duration of viral transduction depends almost entirely on the nature of the vector. Truly permanent transduction requires insertion of vector DNA into host chromosomes, which can be accomplished with retroviral vectors based on lentivirus or similar agents [40]. As is well known, such vectors pose a small but non-zero risk of disrupting oncogenes and setting the stage for tumor formation [41]. Safer vectors include those based on adenovirus (Ad), including the classic E-1 deleted vectors [42] along with the more refined helper-dependent adenovirus (HDAd) [43] and adeno-associated virus (AAV) [44] constructs. These non-inserting vectors sustain transduction by persisting as non-replicating episomal elements in the host cell cytoplasm. When the cell divides, however, vector DNA is lost or diluted. Therefore transgene transduction depends on the lifespan of the target cell, which is unlimited in neurons, cardiac and skeletal muscle cells, quite brief in most epithelia and smooth muscle, but moderately long in liver (1 to 3 years depending on species), [45,46].

Drawbacks and obstacles to gene transfer

One major present drawback is a persistent concern for safety, which is well-founded and deserves attention. A key risk factor appears to be the brief but intense innate immune response to viral capsid proteins [47]. Much research has concentrated on this issue and how to minimize or avoid it [48,49]. Deletion of multiple viral genes while preserving transduction capability is one important step, as in the engineered helper-dependent adenoviral vectors [50] or the vectors based on naturally helper-dependent adeno-associated virus [51,52]. These modified agents typically have no ability to express viral proteins in vivo and thus, longer term, may remain "hidden" from immune surveillance [53]. On the

other hand, to deliver an engineered DNA payload, viral particles must first attach to the plasma membranes of host cells that can translate the genetic information into a protein product. This function requires packaging of the functional DNA within a coat that a) protects it from dispersal and metabolic attack, b) selectively binds surface targets that are characteristic of the selected host cells, and c) stimulates endocytosis of the delivered DNA. At the present state of vector technology the most efficient means of accomplishing this objective uses artificial protein coats derived from the shell of a relatively benign “helper virus”. The immune system still recognizes these coats, which remain able to trigger a seriously adverse response. Such responses are typically short-lived, however. They are also dose related and can be minimized by delivering a smaller load of vector, albeit at the price of obtaining less transgene product.

Fortunately, transient immunosuppression with anti-inflammatory steroids, such as dexamethasone, will reduce host immune responses to viral vector and lessen subsequent toxicity [54] while also prolonging transgene expression [55]. Often, immunosuppression is also required for adequate levels of therapeutic protein. This is especially true in animals subjected to procedures creating inflammatory stress (e.g., implantation of multiple cannulae), which can impair subsequent virally-mediated transgene expression (Gao and Brimijoin, unpublished data). In higher organisms, especially non-human primates and humans, the acute immune response to vector is very strong, associated with a sharp rise in cytokines such as interleukin 6, while immunosuppression is more difficult and less effective [56]. Progress in this arena is critical to future success.

Accelerated Metabolism

As noted initially, accelerated drug metabolism is developing as an alternative way of reducing cocaine access to the brain, a key step in the reward process that generates and maintains addictive behaviors. This approach is tenable only because of butyrylcholinesterase (BChE), a single plasma enzyme that converts cocaine into virtually inactive metabolites, benzoic acid and ecgonine methyl ester [57]. In contrast, the metabolism of amphetamines, nicotine, and many other stimulant drugs depends on cytochrome-p450-dependent pathways in the liver [58,59]. These pathways require intact electron transport chains that only operate in an intracellular environment and cannot readily be enhanced by an exogenous enzyme. As a potential therapeutic agent, BChE appears very safe. Like acetylcholinesterase (AChE) it does hydrolyze acetylcholine, but it plays little if any role in cholinergic transmission or any other known physiological process. Also, as a natural constituent, BChE accompanies every plasma transfusion without discernible effects. In view of these facts, Gorelick in 1997 proposed using human BChE to treat cocaine overdose [60]. Because natural BChE is not highly efficient with cocaine, Lockridge and collaborators tried site-directed mutagenesis and obtained a BChE with 4-fold improved catalytic activity [61]. This process, aided by computer-based modeling of cocaine docking and catalysis, continued in other laboratories, including ours and that of Chang-Guo Zhan, leading to a series of “rationally designed” BChE mutants with increasing catalytic activity against cocaine [62–66]. The key enzyme yardstick is “catalytic efficiency”, defined as the ratio between “k_{cat}” (molecules of substrate hydrolyzed per min, per active site) and “K_m” (substrate concentration at half-maximal velocity). Unless drug saturates the binding site (an improbable condition) an efficient enzyme will out-perform others with higher catalytic “power” (larger k_{cat}) but disproportionately higher K_m. Compared with natural BChE, the optimal mutant cocaine hydrolase, “CocH”, is less efficient with acetylcholine but 1300-fold more efficient with cocaine [66].

The advent of catalytically efficient CocH led to the finding that an enzyme can not only prevent cocaine toxicity in animal models but also reverse it. In fact CocH is able to abort lethal cocaine-induced seizures in rats and reliably rescue them even when delivered after

major convulsions have already began [67]. Even more interesting are findings indicating that pretreatment with CocH prevents cocaine-primed reinstatement of cocaine-seeking behavior in rats trained on cocaine self-administration [68]. That outcome pointed toward a possibility of using enzymatic therapy to aid recovering addicts avoid relapse into drug taking. Encouragingly, unmodified BChE had proved remarkably benign in studies undertaken by the US Department of Defense for prophylaxis against chemical warfare agents. In fact, rodents showed no ill-effect after treatment with BChE in doses that raise plasma levels hundreds of fold [69–71]. Human subjects given gram quantities of the enzyme also experienced no perceptible changes, subjective or objective. In this sense, BChE seems “physiologically inert”. One can speculate that it evolved for metabolic disposition of bioactive esters in the diet. In our opinion, however, direct administration, even of a safe and effective esterase is still problematic for the chronic treatment needed with addiction. Expensive and highly purified protein would have to be given frequently in large amounts. Even slow-release preparations would often need renewal. Costs could be exorbitant and patient compliance, problematic. These facts led us to consider delivering BChE-based CocH by therapeutic in vivo gene transfer, either as a “stand-alone” treatment for relapse-prevention, or as part of a combination therapy with anti-cocaine vaccine.

Cocaine hydrolase gene transfer with helper-dependent adenoviral vector

To date, several animal studies have been completed with a view toward assessing the safety and efficacy of CocH gene transfer. Early studies were based on a first-generation E-1 deleted adenoviral vector. The results in rats showed very high levels of transgene expression, elevating cocaine hydrolase activity in plasma by an average multiple of 50,000 [72]. Such levels were only sustained for a few days, however, limited by a strong host response to transgene and, probably to transduced hepatocytes as well. To achieve more stable transduction, our group has utilized two newer generation vectors: first, a helper-dependent adenoviral vector (HDAd) based on constructs developed by Parks, Ng, and others [43,73,74]; and more recently, an adeno-associated viral vector [75,76].

The viral coat of the hdAD vector is similar to the older vector, as is the initial immune response. However, lacking DNA for all viral proteins, hdAD does not provoke sustained immunological reactions. This vector is a clear advance and has impressive ability to sustain long-term transduction in liver. Thus, Ng and collaborators obtained high circulating levels of human alpha antitrypsin for over one year in baboons [50] and lifetime expression (~2.5 year) of the ApoE gene in genetically deficient mice [77]. Our strategy also targets liver as the locus for gene transduction, given that this organ is the natural site of BChE synthesis and release into plasma, and that its high blood flow ideally suits it for metabolic surveillance. In addition, hepatocyte lifespan, both in rodents and humans, should permit effective transduction of enzyme for a considerable period of time.

Current work has demonstrated that gene transfer of CocH with a liver-directed HDAd vector can sustain very high levels of circulating enzyme in rodents. Specifically, we obtained 1000-fold increases of cocaine hydrolyzing activity for at least six months in rats [68]. In separate and ongoing studies, mice are showing substantial hydrolase transduction 18 months after initial treatment [Gao, Geng and Brimijoin, unpublished results]. Therefore continued testing is focused on hepatotropic viral vectors incorporating liver-specific promoters. Signs of toxicity have been absent at the doses used (up to 10^{13} particles per mouse, delivered through the tail vein), which elevated circulating cocaine hydrolase activity by a factor of approximately 1,000,000 [78]. In particular, blood monitoring for liver enzymes has detected no elevation (implying continued integrity of hepatocytes), and liver tissue has appeared entirely normal in post-mortem histological sections. Treated mice show normal behavior patterns, normal levels of spontaneous activity and weight gain, and when challenged with doses of cocaine that are hepatotoxic or lethal in unprotected animals, they

show dramatically reduced toxicity or none at all [78]. Rats given a single vector injection also showed no sign of adverse effect. However, during the entire six month period of observation, the treatment blocked the drug-seeking "reinstatement behavior" usually triggered by cocaine exposures [68]. Somewhat surprisingly, a retrospective analysis of the data revealed that this failure of "cocaine-primed reinstatement" persisted even in the few rats that lost cocaine hydrolase expression after 3 to 4 months. That effect was specific to cocaine. Thus, at 6 months, the same rats showed robust drug-seeking after a priming injection of amphetamine, a stimulant not metabolized by CocH. A possible explanation for persistent loss of cocaine-primed reinstatement behavior is that vector-treated rats *unlearned* the association between cocaine intake and a drug-related reward. Wise and colleagues have provided convincing evidence that organisms associate drug-rewards with "interoceptive cues" from peripheral chemoreceptors [79]. These cues are relayed to the brain by fast neural pathways and initiate a release of glutamine in the ventral tegmental area. The glutamate "spike" is followed by a wave of dopamine release caused by drug acting directly on central reward nuclei. In subjects with abundant CocH, the interoceptive cues may persist but unaccompanied by a central reward. Multiple repeats of this experience might eventually extinguish drug-seeking behavior triggered by drug exposure. If the metabolic products of cocaine, namely benzoic acid and ecgonine methyl ester, had mildly aversive properties, that effect could be even stronger. We have no answer to this question as yet but consider it worth exploring.

Combining gene transfer and vaccination

Anti-cocaine vaccine and cocaine hydrolase gene therapy deserve to be considered together because they are different means to the same end: preventing an addictive drug from reaching its principal site of action. In addition, these two different modes of drug interception are compatible with one another and should be mutually reinforcing. Several factors contribute to this potential. Vaccination can easily generate specific antibody titers in the low micromolar range, implying a capacity to bind a major fraction of a typical reward-level drug dose. Since antibody binding occurs almost immediately [80], an initial pulse of drug is blunted. But a quickly repeated or larger dose can still exert central effects because anti-drug antibodies are limited in abundance and therefore saturable. In comparison with vaccination, gene transfer of enzyme is likely to generate fewer drug-binding sites, with lower affinity. However, catalytic action can eliminate virtually any amount of drug and restore a normal state within minutes. In an in vitro model, when CocH was added to a 1 μM solution of cocaine >90% bound with nanomolar affinity to anti-cocaine IgG, the enzyme destroyed 98% of all drug within 90 sec [81]. This reaction is easily fast enough for physiological relevance.

More stringent tests of CocH have been carried out in animal models. In mice challenged with large doses of cocaine, at or near the LD50 (i.e., 100 to 120 mg/kg, i.p.) single treatments with anti-cocaine antibody (8 mg/kg) or CocH (1 mg/kg) did not reduce motor dysfunction and provided only modest protection of liver structure. Given together, however, the same treatments effectively prevented all these toxic actions of the drug [78]. Similar cooperativity was noted with regard to cocaine-induced locomotor activity in rats [82]. The subjects were given KLH-norcocaine vaccine approximately 6 weeks before testing, and a booster injection at 3 weeks, or they received HDAd vector with cocaine hydrolase cDNA approximately 2 weeks before testing. Under the experimental conditions individual treatments reduced cocaine stimulation modestly, while combined treatments provided much greater reduction. To prove that such results reflect truly synergistic actions will require rigorous investigation of a broader range of treatment levels, along with isobolographic analysis. Meanwhile, the benefits are obviously at least additive. Therefore, anticipating future therapy of human drug users, we envisage dual treatments by vaccination

and cocaine hydrolase gene transfer. The primary goal would be to provide more robust protection from addiction relapse than could be obtained from either treatment alone. In addition, combined treatments might permit reduced vector dosages, and less frequent booster immunizations, resulting in increased safety, lower costs and reduced inconvenience.

Potential problems and pitfalls with cocaine hydrolase gene therapy

A general limitation of gene therapy, apart from the risks of toxicity already discussed, is related to acquired immunity directed against the specific viral antigens presented by the particular gene transfer vector to be used. This problem arises in two forms. First, if a subject has previously been exposed in daily life to a virus of the same serotype as the vector, active immunity will already be in place and the initial transduction will fail. This event may pose some risk to the recipient and it will block future attempts at gene transfer with that vector. For the same reason, when a subject experiences successful transduction but needs to prolong it, re-administering the same vector will be ineffective. Therefore, pre-treatment screening for viral protein antibodies will be essential. Fortunately, however, work in animal models indicates that “serotype switching” or use of a vector based on a different virus is an effective solution [83].

Another question is whether CocH transduction might arouse immune responses that curtail the therapeutic effect. The sustained high levels of cocaine hydrolyzing activity in liver and plasma after viral gene transfer in rats imply that the mutated human BChE was tolerated by their immune system, at least to a degree. Nonetheless, looking further into the issue, we tested directly for anti-BChE IgM or IgG in rat blood samples at multiple intervals after transduction. None were detected at 3, 20 and 30 weeks. A few animals tested weakly positive at 50 weeks, but CocH serum half-life (measured by recovery from irreversible inhibition) was not shortened [84]. We concluded that CocH is only weakly antigenic in this species.

The long duration of HDAd-mediated CocH transduction is partly due to the lack of encoded DNA for viral proteins. This advantage is shared by AAV-based vectors, the most commonly used gene transfer agents in recent clinical trials. Ongoing studies in our laboratories are investigating AAV for CocH transduction in mice. Mice appear somewhat less tolerant to CocH than rats. When transduced by low-dose AAV, type 2/8, mice develop antibodies to human BChE and lose most of their transgene expression within two weeks (Gao and Brimijoin, unpublished data). Immune reactions to native BChE should not occur in human subjects other than those rare individuals with a null gene mutation [85,86]. However, the occurrence of anti-BChE antibodies in rats and mice raises the question whether CocH, as a BChE mutant, could be weakly antigenic in humans. Our hypothesis is “no”, because all mutations are confined to the catalytic gorge and at least partly hidden from immune surveillance. This hypothesis deserves to be tested before working toward an eventual human trial. For that purpose comparable mutations were made in mouse BChE, and immune responses were examined after transduction with HDAd or AAV. Unpublished results in both cases indicate enzyme persistence for months with complete absence of antibodies to the transgene product. Because mutating the key sites in a conspecific enzyme does not confer immunogenicity, it seems likely that the equivalent mutations in human BChE will also be “immunologically silent”.

One theoretical concern regarding greatly elevated plasma CocH is that this BChE-derived enzyme retains substantial ability to hydrolyze the cholinergic neurotransmitter, acetylcholine [87]. In actuality, the likelihood that CocH will cause problems with cholinergic neurotransmission is remote. Extensive prior research has shown little physiological impact of exogenous BChE. Thus, studies to evaluate human BChE as a

prophylactic treatment for exposure to chemical warfare agents uniformly failed to detect physiological disturbances in rats, guinea pigs and primates, even after administering near-gram quantities that raised plasma enzyme levels 50- to 100-fold [69–71,88–90]. This impressive evidence for safety and the absence of cholinergic dysfunction is counterintuitive but not illogical. There are at least two reasons. First, mammalian blood contains approximately equivalent molar concentrations of AChE (in plasma and red cells and BChE (plasma only), but the former is over twice as catalytically efficient with acetylcholine [91,92]. Hence, a 10-fold increase in circulating BChE only triples hydrolysis capability. More important, motor synapses are all packed with cholinesterase at levels thousands of times above those in the general circulation or extracellular water. Elegant morphometric studies by Anglister, Salpeter, and colleagues [93] provided a reliable basis for quantifying actual AChE concentrations within the synaptic cleft of the neuromuscular junction. With a snake neurotoxin serving as a highly selective and high affinity probe for this enzyme (^{125}I-labled fasciculin) these investigators established that the density on the synaptic membrane was 5×10^{19} catalytic subunits per ml, or roughly 10^{-4} M. By contrast, published values indicate the concentration of BChE subunits in normal mouse serum to be about 2×10^{-9}M. Therefore, considering relative catalytic efficiencies it would require at least a 100,000-fold increase in plasma BChE levels to match the ACh-hydrolyzing capacity of motor synapses. Thus, even the upper limits of plasma BChE expression are highly unlikely to affect transmitter levels or duration at motor synapses. Cholinergic synapses in the brain are also unlikely to be disturbed as they are well insulated from circulating hydrolase by virtue of the blood brain barrier, which excludes BChE [94]. Among the few remaining areas for concern are the nicotinic synapses in the peripheral autonomic ganglia and, perhaps, muscarinic synapses at end organs innervated by parasympathetic nerves. Investigations of cardiovascular function in vector-treated mice are presently underway.

CONCLUSION

There is exciting potential for broad application of anti-drug vaccines as one component of a comprehensive approach to managing drug addiction, aiding in recovery, and preventing relapse. Vaccines that generate effective drug-binding IgG antibodies at high titer are well along the road to clinical application for treating both nicotine and cocaine abuse. The feasibility of long-term gene transfer delivery of even more effective monoclonal antibodies for the same drugs has recently been established. Finally, in the case of cocaine, metabolic elimination of drug by highly efficient engineered enzymes holds promise as an alternative or complementary strategy for intercepting the stimulant before it reaches brain reward centers.

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ABBREVIATIONS

KLH	keyhole limpet hemocyanin
CTB	cholera toxin
TTX	tetanus toxin
OMPC	outer membrane protein complex of neisseria meningitidis group B bacteria
BChE	butyrylcholinesterase
AChE	acetylcholinesterase
HDAd	helper-dependent adenoviral vectors
AAV	adeno-associated viral vectors
$\mathbf{k_{cat}}$	maximal reaction velocity per enzyme catalytic site
$\mathbf{K_m}$	substrate concentration yielding half maximal enzyme velocity

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EXPERT COMMENTARY

Continuously improving technology should be applied to increase the effectiveness of "classical" vaccines against drugs of abuse. The key to success will be increased titers of high-affinity anti-drug antibodies. Promising avenues to explore include conjugation of drug haptens with more highly immunogenic carrier proteins, such as those derived from adenovirus capsids. Delivering optimized monoclonal anti-drug antibodies by gene transfer over extended periods without need for repeated interventions is another idea well worth exploring. Issues of safety and efficacy with that approach remain significant but are probably surmountable. As the demonstrated and perceived safety of gene transfer improves, this technology can also be applied to deliver drug-metabolizing enzymes. At present the obvious choice for a therapeutic anti-drug enzyme is a cocaine hydrolase based on natural human plasma cholinesterase. Besides having therapeutic potential on its own, such an enzyme is likely to complement and magnify the effects of anti-cocaine vaccines.

FIVE-YEAR VIEW

One can safely predict incremental advances in anti-drug vaccine technology as the choice and design of haptens and carrier proteins are refined. We speculate that in five years' time there will have been strongly positive outcomes from clinical trials of several treatment categories touched upon in this review with regard to cocaine and nicotine abuse. In particular, "classical vaccines" with more effective carrier proteins and adjuvants will have proved able to elicit higher levels of anti-drug IgG than previously seen, with more than one third of the subjects attaining levels associated with a marked reduction of abuse liability. Second, clinical gene transfer of a "humanized" high-affinity monoclonal antibody to nicotine or cocaine will have succeeded in generating still higher levels of anti-drug antibodies and greater in vivo drug antagonism, accompanied by limited and tolerable side effects during the initial stages of transduction. And third, a clinical trial of viral gene transfer of cocaine hydrolase will be in the process of demonstrating safety and a degree of efficacy that, if not adequate in itself, could greatly amplify the therapeutic results of anti-cocaine vaccine.

Key issues in this review

- Vaccines have been developed against multiple addictive substances including cocaine, nicotine, amphetamine, and heroin, with the aim of reducing abuse liability and aiding abstinence.
- Such vaccines are based on the conjugation of drug haptens to immunogenic carrier proteins.
- Existing vaccines, in clinical trial or current use, are moderately effective at best.
- Drug vaccines under development utilize more intensely immunogenic carrier proteins, such as coat proteins from killed adenovirus, to generate higher titers of anti-drug antibodies.
- Under investigation as a possible alternative to conventional vaccines is gene transfer of monoclonal antidrug antibodies engineered for optimal affinity
- Gene transfer of drug-metabolizing enzymes is an alternative “vaccine-like” approach to treatment of substance abuse.
- Recent animal studies involving therapeutic gene transfer of cocaine hydrolase validate the concept of accelerated drug metabolism as an alternate means of reducing cocaine’s abuse liability.
- Current research suggests that a combination of the immunologic approach (vaccines and antibodies) and the metabolic approach (gene transfer of enzymes) is likely to be particularly effective.