Immunology and Cell Biology (2009) 87, 309–314

www.nature.com/icb

REVIEW

Anti-drug vaccines to treat substance abuse

Berma M Kinsey[1,2], David C Jackson[3] and Frank M Orson[1,2,4]

Substance abuse is a growing world-wide problem. The big four drugs of abuse that might lend themselves to immunotherapy are nicotine, cocaine, morphine/heroin and methamphetamine. Tobacco abuse has a well-known enormous impact on major chronic cardiovascular and pulmonary diseases, while the last three, aside from their neuropsychological effects, are illegal, leading to crime and incarceration as well as the transmission of viral diseases. Having an efficient vaccine that would generate antibodies to sequester the drug and prevent its access to the brain could go a long way toward helping a motivated addict quit the addiction. This review will discuss what has been done to bring such vaccines to human use, and what the challenges are for the future of this promising intervention.
Immunology and Cell Biology (2009) **87,** 309–314; doi:10.1038/icb.2009.17; published online 31 March 2009

Keywords: anti drug; drugs; vaccine; substance abuse

Abuse of illegal drugs is a scourge that has been growing in both large metropolitan areas and in rural settings all over the world. Enormous amounts of money continue to be spent by governments on the so-called 'war on drugs', which has been spectacularly unsuccessful.[1] Enormous amounts of money continue to be made by international drug traffickers, and many lives have been lost in battles among these illegal organizations.

Is substance abuse a disease or an infection that could lend itself to prevention or cure by the delivery of a vaccine against the drug of abuse? It could be argued that the propensity for abusing drugs has a genetic basis[2,3] but on the other hand the social and peer pressure attributes of drug taking would make it seem more like an infectious disease. Indeed, it leads to disruptions in the lives of the addicts and in those of their entire community[4,5] just like typhoid or diphtheria infections used to do. Unless a genetic link was definitively pinned down, prevention of drug abuse by vaccination is not an option. However, if an addict conceived a desire to quit the addiction, *post facto* vaccination might prove helpful in breaking the established habit.

The major drugs of abuse, nicotine, morphine (heroin), cocaine and methamphetamine all depend on access to the brain for their psychological and reward effects. An effective vaccine, that is one that produces a high concentration of antibodies with high affinity for the drug, would bind the drug molecule in the circulation and prevent it from crossing the blood–brain barrier and accessing its receptor in the brain. As an example, say a vaccinated ex-smoker went into a room full of cigarette smoke. Most, if not all, of the nicotine inhaled from the second-hand smoke would be sequestered by the circulating antibodies. The amount of nicotine reaching the brain would not be enough to provide the reinforcing effect of the craving sensation that might tempt the person to start smoking again.

All of the anti-drug vaccines that have been developed so far have shown promising results in rodents, both in terms of the concentration of antibodies elicited by the vaccine and in the reduction of drug-associated behavior shown by the vaccinated animals when challenged with the drug.[6–8] Accordingly, vaccines against nicotine and cocaine have gone straight from rodents to clinical trials, while anti-morphine and anti-methamphetamine vaccines are waiting in the wings.

We will first examine the construction of an anti-drug vaccine, followed by a discussion of the theory of antibody binding. We will then look at the individual vaccines that have been prepared and studied, and what needs to be done in the future to follow up on the promising starts that have been made.

CONJUGATE VACCINES

As drugs of abuse are small molecules (Figure 1), on their own they are not capable of eliciting antibody responses. Such small molecules need to be attached (conjugated) to an immunogenic protein or other structure (a carrier) to elicit an immune response. The drug molecule is usually functionalized with a linker sequence that allows conjugation to the carrier. The linker can be covalently attached to the drug molecule in various places depending on the drug's chemical structure, and various chemistries can be used to attach the so-called hapten to the carrier. The conjugated carrier is purified so as to remove any free hapten and mixed with an adjuvant such as Freund's complete adjuvant for animal use or alum for human use. Delivery of the finished vaccine is often done intraperitoneally in animals for

[1]Research Service, Michael E DeBakey Veterans Affairs Medical Center, Houston, TX, USA; [2]Department of Internal Medicine, Baylor College of Medicine, Houston, TX USA; [3]Department of Microbiology and Immunology, The University of Melbourne, Parkville, Australia and [4]Department of Immunology and Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX, USA
Correspondence: Dr BM Kinsey, Department of Internal Medicine, Baylor College of Medicine, MEDVAMC, Room 221, Building 109, MEDVAMC, 2002 Holcombe Blvd, Houston TX 77030, USA.
E-mail: bkinsey@bcm.tmc.edu
Received 3 February 2009; accepted 1 March 2009; published online 31 March 2009

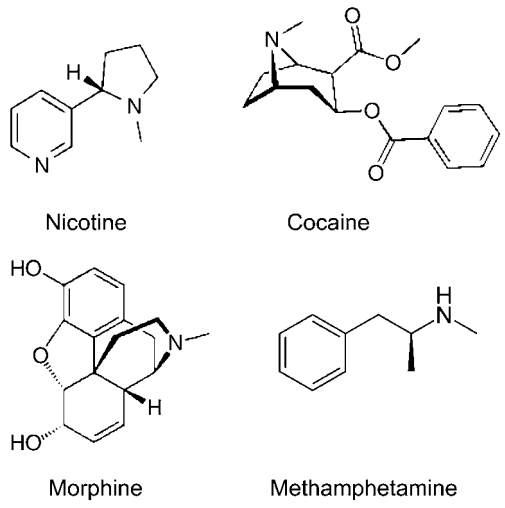

Figure 1 Chemical structures of the major drugs of abuse.

convenience but is usually done intramuscularly in humans for better acceptance. As exposure to the drug itself will not serve to boost the immune response, a schedule of delivery is devised so that the concentration of antibody remains at a suitable level. Oftentimes many different conjugate vaccines are prepared, based on different drug-linker structures, different attachment chemistries and different carrier proteins (nine in the case of nicotine),[9–17] and tested in rodents before one is selected for human use. A proposed vaccine should have no adverse side effects, and the antibodies should be produced at a high level, sufficient to bind most of a pharmacologically active dose of the drug. The antibodies should also have high affinity and high specificity for the native drug of interest; that is they should bind strongly and preferentially to that drug rather than binding to other compounds, especially not to the inactive drug metabolites that might otherwise compete for antibody binding sites. It would be desirable to have little antibody binding to the linker moiety as well, because these antibodies would likely bind poorly, if at all, to the free drug in the circulation.

ANTIBODY BINDING THEORY

The effectiveness of a drug vaccine will be inevitably tied to the quantity and quality of the antibodies that the vaccine can elicit. Ideally, an effective vaccine will rapidly elicit large quantities of antibody that can successfully bind the drug in the circulation, and prevent its movement to the brain where the most relevant pharmacological effects are mediated. Simple blockade of the drug molecules has a conceptual attraction, but other issues become important in drugs of abuse, because different drugs may be used in markedly different doses, or in different patterns of administration. Cocaine and nicotine provide an example of this contrast, in which the former is most often used intermittently, while the latter is usually administered almost constantly in higher doses through smoking cigarettes or using other tobacco products. It is nonetheless conceptually useful to consider a simple blockade effect by antibodies that will obviously be directly dose dependent for single administrations, because at a sufficiently high dose of the target drug, any amount of antibody could become saturated so that the residual drug would spill over and behave pharmacologically as if no antibody were present at all. Using this simple model, it is possible to determine how much antibody of a defined binding quality (affinity) would be required to block a typical dose of an abused substance. Cocaine has been well studied in this regard, and the calculations are discussed in the following paragraph.

Laboratory studies of cocaine addicts who were off the drug long enough to become negative by urine testing showed that doses of cocaine sufficient to achieve peak plasma concentrations of 0.5 μM elicited both the physiological and psychological effects typical of cocaine use.[18] With some reasonable assumptions (discussed below), the serum concentration of immunoglobulin G (IgG) antibody required to bind a significant fraction of this amount of cocaine can be calculated directly, using the law of mass action:

$$A+B \underset{k_r}{\overset{k_f}{\longleftrightarrow}} AB \quad Ka = \frac{[AB]}{[A]\,[B]}$$

In these equations, AB represents the bound complex of molecules A and B, and Ka represents the binding equilibrium constant, the ratio of the forward binding rate constant k_f and the reverse, or dissociation rate constant k_r. Although IgG has two binding sites and thus a more complex behavior than is represented by this equation (discussed in detail elsewhere[19]), the fact that drugs are small molecules that will bind each site on the IgG antibody independently nonetheless allows this equation to provide a reasonable estimate for the average binding behavior of antibodies and drugs in this context. Small molecule binding to antibodies has been thoroughly studied, and a number of useful observations for this discussion are now well established. First, initial antibody binding in solution to a small molecule target (the forward reaction) is very rapid in a well-mixed solution, occurring in less than a second.[19–21] As a result, the affinity constant is most strongly influenced by the dissociation reaction. Typically, the affinity of a primary immune response in the first few weeks of antibody development is in the 1 μM^{-1} range,[22] which is the intrinsic affinity of the individual binding sites for most IgM and early IgG antibodies. With affinity maturation, IgG antibodies achieve higher intrinsic affinities ranging typically from 20 to 200 μM^{-1}, although some antibodies may bind much more tightly. For a conservative estimate of the amount of IgG antibody with an estimated average affinity of 30 μM^{-1} required for binding 80% of a 0.5 μM concentration of cocaine, the calculation predicts a total specific antibody binding site concentration of 0.53 μM (in more conventional terms, about 40 μg ml^{-1} of IgG antibody). However, as mentioned above, the behavior of IgG with two antibody-combining sites is more complex than the binding of a molecule with only one combining site. Binding to the second site with the first site already occupied behaves with a lower net affinity (Ka/2), due to potential for dissociation from either the first or the second combining site.[19] As a result, the actual antibody concentration needed to bind 80% of the drug would need to be somewhat higher than calculated. Nevertheless, given that the antibodies in a polyclonal response would have a wide range of actual affinities, these calculations provide a reasonable estimated range for the antibody response requirements.

NICOTINE

Vaccines against nicotine have progressed the farthest along the pipeline among any of the vaccines against the drugs of abuse mentioned above. Nicotine abuse through cigarette smoking is a major cause of mortality and morbidity worldwide. It is believed that smoking just one cigarette can cause some individuals to become 'hooked'.[23]

As cigarettes are acquired legally, and the perception is that nicotine is a more or less harmless recreational drug that does not interfere

with everyday functioning, stopping smoking is a daunting prospect for the addict who really wants to quit. There has been some success in trials involving the use of nicotine replacement therapy (patches, gum and others) with or without the addition of denicotinized cigarettes, and drugs such as mecamylamine and varenicline that bind to nicotinic receptors have also shown some efficacy.[24]

However, all these interventions require essentially daily compliance on the part of the smoker, and it would seem that vaccination might offer a better option, in that antibodies persistently present in the circulation could sequester the nicotine and substantially reduce the amount of drug reaching the brain and delivering its pleasure and positive reinforcing effects. Three companies have completed phase I and phase II clinical trials with different vaccine constructs: Nabi Pharmaceuticals with NicVax, Cytos Biotechnology with NicQb and Xenova Group with TA-Nic.[25] These companies relied on different carriers but attached the nicotine molecule to the carrier through a linker bound to a 3′-hydroxymethyl or 3′-aminomethyl group on the nicotine nucleus. In all cases the vaccines were well tolerated, and the people with the highest levels of antibodies were more likely to be able to abstain from smoking, in some cases for as long as a year. The results of the trials were somewhat disappointing in that there was a large variation among the trial participants in the amount of antibody generated, and only a relatively small percentage of the participants managed to stay away from smoking. Nevertheless, it is expected that the three companies mentioned above will try to bring their vaccines to the market between 2009 and 2011.[26]

In spite of this, nicotine vaccine research continues apace. One group recently published a study of a bivalent vaccine, one where two different nicotine-linker constructs were separately conjugated to two different carriers and administered together. The bivalent vaccine gave significantly better results than each individual vaccine alone. Another group made a vaccine using a new nicotine-linker combination, which was also used successfully. One would hope that such efforts will lead to improvements in anti-nicotine vaccines that will lead to better outcomes for the nicotine addict.[27,28]

COCAINE

The abuse of cocaine has penetrated all levels of society in the developed world, and it is a growing problem in underdeveloped nations. Drug cartels regularly engage in wars, with great loss of innocent lives. Where injection is the favored way of delivery for this drug, it leads to the spread of infectious diseases, including hepatitis and AIDS.[29] Cocaine is a very seductive drug, and it has been used by indigenous peoples for centuries in South America, where the coca plant is easily grown. Merely chewing coca leaves allows one to work at high altitudes, and purified cocaine, especially the free base ('Crack') is easily smoked or ingested, leading to feelings of potency and euphoria. Abuse of the drug interferes with leading a productive life; as it is illegal, the user often falls into crime to support an increasingly expensive habit. Unlike the case with nicotine, where pharmacological interventions have been used with modest success, there is nothing really satisfactory available for cocaine along those lines.[30–32]

For those addicts who have a desire to stop abusing cocaine, vaccination would seem to offer some benefits, especially in conjunction with counseling and therapy. The same caveats would apply for a successful cocaine vaccine as for a nicotine one: the vaccine should have few side effects, and should elicit high levels of antibodies of good affinity after a reasonable delivery schedule. The cocaine molecule offers several sites for attachment of a linker structure. The methyl group of the methyl ester group can be removed and another ester or amide which serves as a linker put in its place, with minimal change to the shape and charge of the molecule.[33] Similarly, the benzoyl group can be replaced with a substituted benzoic acid whose substituted group can be used to form an attachment to a carrier.[34,35]

The methyl group on the nitrogen can be removed, and a linker group attached there. If that linker is a carbon chain, the charge on the cocaine molecule will be little impacted. If an amide is formed at that position, as would be the case with a succinyl group, the charge on the molecule, which may play a role in antibody binding, will no longer be there.[7] In 1997, a novel method was studied where a photoactivatable group was attached to keyhole limpet hemocyanin (KLH). UV irradiation resulted in the photoactivatable group inserting randomly in the cocaine molecule. This vaccine did give production of antibodies in rodents.[36] This particular method was not pursued further, but all of the other ways of attaching a linker to the cocaine molecule so as to prepare a conjugate vaccine have been used with various carrier proteins, and most have been tested successfully in rodents.

The only anti-cocaine vaccine carried forward in human clinical trials so far has been one where succinyl norcocaine was attached to recombinant cholera toxin B by standard chemical methods. Similar to the nicotine trials, there was a large disparity among the participants as to the antibody concentrations achieved, and the high responders had better success at staying off cocaine.[37] It is not known exactly what was the reason for the disparity in antibody levels, but the 25% of the participants who had substantial levels of anti-cocaine IgM antibodies in their serum before vaccination had low levels of IgG antibodies after vaccination. Deng *et al.*[38] previously reported detection of antibodies against cocaine in unvaccinated addicts, and covalent conjugates of cocaine and normal serum proteins were detected in those individuals. Whether in this case the presence of IgM antibodies could account for the relative lack of response to vaccination is not known.

The approximate concentration of cocaine in the blood after a session of smoking crack, for example, is well known,[18] and the concentration of anti-cocaine antibodies in vaccinated individuals is also known. Comparing those numbers makes it evident that a person determined to get a high from cocaine could easily just take more. That is why it is so essential that anti-cocaine vaccination be accompanied by other forms of intervention, such as drug counseling, to achieve a successful outcome for the addict.[39]

MORPHINE

Morphine is a drug commonly used for analgesia on the battlefield as well as in the hospital. It is extracted from the latex coming from the seedpods of the poppy plant, which is easily grown in the mountains of Afghanistan. From there and from the 'Golden Triangle' in Southeast Asia this drug, and its readily made and more potent derivative diacetyl morphine (heroin) have flooded the world. In the early 1970s, vaccines against morphine were created and tested by several groups.[40,41] The most useful one was from 6-succinyl morphine, because the antibodies that were generated bound not only morphine, but heroin and 6-acetyl morphine as well. This is desirable because it is well known that the widely abused drug heroin, a derivative of morphine, is rapidly metabolized to the pharmacologically active compounds 6-acetylmorphine and morphine. This early work was recapitulated in 1999, and again in 2006 by a different group using a slightly different method.[42,43]

At about the same time as the anti-morphine vaccines were being studied in the 1970s, pharmacological agents such as methadone were developed for opiate addiction and successfully introduced in

Western countries,[44] so that vaccine interventions fell by the wayside. However, interest in anti-morphine and -heroin vaccines has been rekindled in the developing world, where methadone treatment has proven too expensive or is not well accepted by addicts. As these opiates are usually injected, and HIV infections are spread by the use of 'dirty' needles, the health services of many countries such as China and Mexico are considering vaccines as a way of curbing opiate addiction and thus slowing the spread of the AIDS epidemic. The 1970s morphine vaccine was well studied in rhesus macaques as well as in rodents, and therefore it would seem to be poised to be applied to humans, although no clinical trials have been started as yet.

METHAMPHETAMINE

Methamphetamine addiction is probably the most terrible of all addictions in its effects on the lives of the addicts and those around them. It is a highly addictive stimulant, has a longer half-life than cocaine and is partially metabolized to amphetamine, itself a powerful stimulant. A criminal enterprise has grown up around the synthesis of this fairly simple molecule (Figure 1). The synthesis can be carried out in house trailers using readily available starting materials, and there is much money involved in its production and sale. Methamphetamine addition is different in that methamphetamine is readily available in the rural areas where it is made, rather that in the urban settings where heroin and cocaine predominate.

The development of anti-methamphetamine vaccines for human use has progressed less far than for the other vaccines discussed, but this does not mean that there has been no interest in the field. Several groups have used vaccination to produce therapeutic monoclonal antibodies against methamphetamine and some of its closely related derivatives such as amphetamine and 'Ecstasy' (methylenedioxy methamphetamine).[45–48]

Such monoclonals could presumably be useful for treatment of overdoses, or could be used in a clinical setting to aid cessation of methamphetamine abuse. Some pharmaceuticals have been proposed as replacement therapies to blunt the psychological effects of methamphetamine use, but their side effects are generally undesirable.[49,50]

The fact that monoclonal antibodies have been produced indicates that anti-methamphetamine vaccines are immunogenic, but these have not been studied *per se* as agents for therapeutic vaccination as is the case with the other anti-drug vaccines. In our laboratory, we have produced conjugate vaccines against methamphetamine that elicit high-titer antibodies in vaccinated mice. We found that when vaccinated animals were given doses of methamphetamine similar to those found in drug addicts, the inhibition of methamphetamine-stimulated locomotor activity roughly corresponded to the amount of antibody in the serum of individual animals, strongly suggesting that vaccination could prove useful for human addicts who were motivated to cease using this highly addictive drug (FM Orson, unpublished). The development of methamphetamine conjugate vaccines is ongoing in our laboratory.

NONTRADITIONAL VACCINE CONSTRUCTS

A strategy being developed by us involves the design of completely synthetic, self-adjuvanting epitope-based vaccines.[51]

In this approach, vaccines are assembled in which the target epitope, which is either antibody inducing or cytotoxic T-cell inducing, is coupled to a helper T cell (T_H) epitope with the lipid moiety dipalmitoyl-S-glyceryl-cysteine (Pam2Cys) situated between the two epitopes. A schematic of this arrangement is shown in Figure 2.

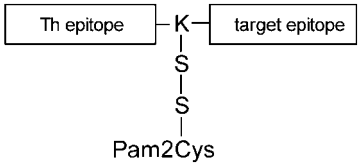

Figure 2 Schematic of the organization of a synthetic self-adjuvanting lipopeptide vaccine.

Candidate antibody-inducing vaccines based on this structure that contain epitopes of luteinizing hormone releasing hormone,[51,52] gastrin[51] or a neutralizing epitope of group A streptococcus[53] have all been assembled and tested successfully in animal models. In addition, vaccines based on epitopes that induce cytotoxic T lymphocytes against influenza virus, *Listeria monocytogenes* and tumor antigens have also been successfully evaluated in various animal models.[51] In the case of the antibody-inducing immunogens, the efficacy of the lipidated vaccines rivals that of vaccine administered with complete Freund's adjuvant but without the disadvantages of this toxic adjuvant.

The generic vaccine structure described here is designed to target minimal antigenic epitopes to dendritic cells resulting in specific immune responses, and could represent a member of a powerful new arsenal of vaccines. As can be seen, various drug-linker constructs can easily be attached to the lipopeptide moiety by standard chemistries using a peptide synthesizer to form a novel vaccine for any drug of abuse. We have now prepared lipopeptide vaccines against amphetamine and cocaine, which gave satisfactory levels of anti-drug antibodies in mice. This novel anti-drug lipopeptide vaccine construct would be inexpensive to produce, and would not require the presence of an adjuvant to enhance its immunogenicity.

CONCLUSIONS

Vaccination as a tool in the elimination of drug abuse would seem to have much promise. However, the vaccines against nicotine and cocaine that have undergone clinical trials have not achieved complete success. There are several areas which need further study and development.

(1) The drug-linker construct should be optimized as regards to both the placement of the linker on the hapten molecule and the nature of the linker itself. The antibodies elicited by the vaccine should bind preferentially to the free drug rather than to the drug-linker molecule or to the linker itself.
(2) The chemistry of the attachment of the hapten to the carrier should be carefully considered, as it could cause changes in the carrier which could affect the immune response to the hapten.
(3) The number of haptens per molecule of carrier, which has not always been reported in published studies, should be examined for its effect on the efficacy of the vaccine.
(4) The selection of the carrier should be carefully considered. So far, various carriers ranging from simple proteins such as bovine serum albumin, to large protein assemblies such as KLH, to virus-like particles, have been used without really determining if there is a difference in their efficacy. We have discovered this to be the case (FM Orson, unpublished), as some carriers have adjuvant properties that enhance the level of the immune response as well as the speed with which antibodies are produced. Whether previous immunity to the carrier is detrimental or helpful for the production of anti-drug antibodies should also

be studied. If there is a detrimental effect, a vaccine with a different carrier might be used in individual cases.

(5) Adjuvants other than alum are being considered for approval in the United States. One of these adjuvants might be desirable in combination with a particular drug vaccine to enhance the immunogenicity of the vaccine, or lessen the side effects of the injection.

(6) Other modes of delivery for a drug vaccine, such as intranasal or aerosol administration, should be considered.

(7) The dosage of the vaccine and the timing of the vaccinations should be optimized as these may be different for different carriers.

(8) The success of a vaccination strategy for drugs of abuse depends a great deal on the motivation of the abuser and the support system available. Combining vaccination with counseling is seen as crucial for success and combining vaccination with pharmaceutical interventions where appropriate might also be helpful.

Vaccination against drugs of abuse is a strategy which is just starting to come into its own, but its future looks bright.

ACKNOWLEDGEMENTS

This study was supported by the Department of Veterans Affairs (VA) Merit Review Program.

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