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Immunotherapy Against Drugs of Abuse

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Current treatments for drug addiction involve classical pharmacological therapy, involving the use of competitive or noncompetitive agonists (full, partial, or inverse) and antagonists. Drugs of abuse enter the brain after crossing the blood-brain barrier rapidly and binding to the proper receptor(s). They are able to do so because they are small and lipid soluble, and produce reinforcing effects by increasing levels of dopamine in brain areas associated with reward. This occurs in specific systems associated with addiction. In the mesolimbic system, neuron cell bodies originate in the ventral tegmental area (VTA) and project to the nucleus accumbens (NAc), amygdala, hippocampus, and prefrontal cortex. Dopamine is subsequently released, which contributes to the development of addiction. Illicit drug use has cost over $11 billion in health care and $193 billion overall in the United States. Heroin, cocaine, and prescription opioid abuse has become more prevalent in recent years, causing over 6,000 deaths in the U.S. annually (NIDA 2015). Recent literature has begun to conceptualize drugs of abuse as toxins, which has led to new approaches in drug research for the treatment of substance abuse.

One of these approaches is immunotherapy, which integrates vaccination of a drug target and incorporates the body's adaptive immune system as a defense against drugs of abuse. The adaptive immune response is a mechanism of the immune system that is divided into two parts: humoral and cell-mediated immunity. Humoral immunity refers to the circulation of antibodies in the blood to find foreign invaders and free molecules that evoke immune response, known as antigens. Cell-mediated immunity engages T-lymphocytes to recognize antigens and designate infected cells. Antigen presenting cells (APCs) are phagocytic cells that break down antigens into smaller molecules and present them on their cell surface. APCs migrate to lymphatic tissue, which include B cells and T cells. T cells link to the antigen presented on the APC and become activated. This interaction provokes B cells to generate plasma cells that can create specific antibodies and immunological memory (Slonczewski and Foster 2014).

Immunotherapy against drugs of abuse is different from conventional vaccinations against infectious diseases in a few fundamental ways. As stated in the most recent 2005 U.S. patent, the vaccine is directed against a drug molecule, not an infectious particle. Unlike most vaccines, the

primary objective of immunization against addictive drugs is therapeutic, not preventative. However, some clinical objectives regard drug vaccines as practical for prophylactic use, especially in at-risk populations such as adolescents or the fetuses of drug-addicted pregnant mothers. The antigen against which drug vaccines are directed is not itself antigenic, and therefore must be bound to a carrier protein that evokes immune response. Since psychoactive drugs exert their effects when bound to receptors in the brain, they are effectively inactivated due to antibody binding in the periphery. If drug is taken when antibodies are present, the antibodies bind to the drug and prevent its psychoactive and physiological effects (U.S. Patent No. 6,699,474B1). This method of treatment is relatively new and rapidly developing. Its contributions thus far appear promising to aid in breaking the cycle of addiction. By preventing drugs of abuse from entering the central nervous system (CNS), the efficacy of drug effect is reduced.

Conventional treatments with agonists and antagonists are considered "small-molecule pharmacotherapies." However, there are risks to substitution therapy, such as methadone, which is used to treat opiate addiction but may become addictive as an unintended consequence (U.S. Patent No. 6,699,474B1). Immunopharmacotherapy is effective because it blocks the effects of drugs peripherally before they can act centrally (Kosten and Owens 2005) by provoking an endogenous immune response external to the central nervous system, eliminating the concern of neurotoxicity. Neurotoxicity is a consideration in some pharmacological alternatives for the treatment of drug addiction. Antibodies isolate drug molecules in the circulating periphery so that they are designated for neutralization by the immune system.

The 2005 U.S. patent is a continuation of previously filed patents, the earliest dating back to 1991. It states its objections of drug vaccinations by setting primary endpoints for preclinical studies. Antibodies are large molecules (150 kDa) so they cannot cross the blood brain barrier, therefore preventing entry of a drug molecule once it is bound to the antibody (Kosten and Owens 2005). This document reported intentions to increase immunogenicity of previously manufactured drug vaccines by linkage to a carrier protein to improve efficacy against drugs of physical and psychological abuse. Immunotherapies are meant to be applied therapeutically as well as prophylactically, with specificity to one of many abused drugs. The vaccine is prepared by combining an immunogen, such as the drug target bound covalently and ionically to a carrier protein, forming a complex referred to as a hapten. The protein-conjugate is often an immunogenic protein, for example, inactivated cholera toxin is often used as the foreign carrier protein (Kosten

and Owens 2005). The hapten complex is purified by dialysis or chromatography, and then dissolved in physiological solution for injection preparation. This is referred to as active immunization. Adjuvants may also be added to enhance the efficiency of the vaccine, increasing levels of high-quality antibodies in response to immunization. They also enhance the ability of antibodies to destroy the drug target. The addition of adjuvants is significant because clinical trials investigating safety and efficacy of immunotherapy vaccines for abused drugs have reported one-to two-thirds of patients failing to achieve a sufficient antibody response likely do to pharmacogenomics and immune variability. Adjuvants are useful additions to the hapten complex model of vaccination against drugs of abuse since they defend antigen degradation by interstitial fluids, display conformational specificity of the immunogenic complex, and aid in other enhancements of adaptive immune response (Alving et al. 2014). Other kinds of immunotherapy, referred to as passive, involve the introduction of exogenous antibodies produced in another animal, isolated in cell culture and already established as specific to the substance abuse target. These antibodies are often generated from multiple cell lines (Shorter and Kosten 2011).

The effectiveness of immunotherapy vaccines depends on the concentration of antibodies, and is greatest when antibody-to-drug ratio is highest (Pravetoni et al. 2012). Therefore, threshold levels of anti-drug antibodies are required to have an effective immune response. In other words, it is ideal to have maximal concentration and binding affinity in order for immunotherapy to be as effective as possible (Shorter and Kosten 2011). Immune responses are not usually generated against molecules smaller than 10 kDa, which is why antibodies are not normally produced for drugs of abuse, requiring protein conjugation and addition of adjuvant agents in vaccination delivery systems.

Drug metabolites may be used as haptens instead of addictive drug targets since some drugs are rapidly metabolized after absorption but before distribution and delivery to the central nervous system. For example, morphine is rapidly converted into morphine-3-glucoronate and morphine-6-glucoronate (U.S. Patent No. 6,699,474B1). Therefore, targeting the active metabolites of some abused drugs is more effective because they may be present in greater concentrations post-absorption compared to the drug molecule in original form (Kosten and Owens 2005). Two fundamental mechanisms are responsible for the efficacy of immunotherapy to prevent drug action in the brain. One mechanism concerns the binding affinity and specificity of antibodies to immunotherapeutic drug targets to prevent central action. As previously mentioned, increased

concentrations of high quality antibodies that bind with high affinity to a drug or its metabolites is key to the efficacy of this treatment. The second mechanism is slowing the rate of entry of free (unbound) drug molecules in plasma across the blood brain barrier, along with rate of association and dissociation to and from an antibody (Kosten and Owens 2005). These mechanistic considerations are important because they reduce the behaviorally reinforcing effects since a large component of reward is the euphoric "rush" experienced after initial drug administration.

Two types of immunotherapy have been established in preclinical studies concerning abused drugs. Active immunization is a series of vaccinations of drug-protein conjugates to stimulate the adaptive immune response and endogenous antibody production. These antibodies circulate in the periphery and bind to drug molecules. An immunological memory is created by the activation of memory B cells in response to re-exposure by booster shots and subsequent amplification of initial response (Kosten et al. 2014). Passive immunization involves the introduction of preformed antibodies produced in another animal immunized with the drug, and immunoglobulins from serum samples are purified. Polyclonal antibodies are often generated from multiple cell lines. Monoclonal antibodies (mAbs) are preferred for passive immunization because these antibodies are derived from a single cell line and genetically identical to one another (Shorter and Kosten 2011). Importantly, mAbs have exact functional and biochemical characteristics, such as high affinity and specificity to the target drug and/or its active metabolites. These antibodies can be custom-designed and dosing is precise unlike active immunization, which evokes a wide variation of immune responses among individuals (Peterson and Owens 2009) especially if they are immune-compromised at baseline. There are four types of mAbs which vary in efficacy of antigen binding. Murine mAbs are 100% genetically derived from a mouse, whereas chimeric mAbs are a combination of mouse (34%) and human (66%). Humanized mAbs are also a combination of mouse (5-15%) and human (95-85%). Humanized mAbs are 100% human. Chimeric and humanized monoclonal antibodies are often derived from a single cell line and referred to as hybridoma cells since they contain a hybrid of human and murine genetic information in the variable (Fv) region of the immunoglobulin G (IgG) protein. Hybridoma mAb cells are often preferred for passive immunization because their high specificity for the immunological target decreases the risk of cross-reactivity. Monoclonal antibodies are preferred in general though, since introduction of foreign immunoglobulins with the polyclonal method can induce serum sickness and transmission of animal viruses (Kosten and Owens 2005). An immunological memory is not

created in comparison to active immunization, which requires repeated vaccinations. However, the duration of action is much longer in monoclonal antibodies, offering immediate protection by passive immunization.

The clinical objectives of immunotherapy against commonly abused drugs are gaining recognition. These vaccinations are unlike immunizations against infectious agents because intentions are shifted more toward therapeutic intervention rather than prophylaxis. Immunotherapy may be used to treat acute and chronic overdose, reduce drug relapse, and decrease the behaviorally reinforcing effects produced by drugs of abuse by attenuating neural reward system activation. Effective treatments for drug addiction will likely be precipitated by further research in this field, especially for those that do not have currently approved pharmacological therapies but abuse is prevalent, i.e. cocaine (NIDA 2016). However, indications for drug immunotherapy with prophylactic considerations are still meaningful. This method may be implied to reach at-risk groups, including vulnerable adolescents and developing fetuses of drug-addicted mothers (Kosten and Owens 2005). Immunotherapy for preventative application should be further studied concerning immunological drug targets, which range from illicit (heroin) to legal (nicotine).

Preclinical studies have reported promising results from early investigation in the 1970s (Bonese et al 1974) to present day, encouraging drug immunotherapy research and evaluating its efficacy by observing and measuring behavioral effects, self-administration paradigms, and attenuated reward effects. Rats immunized with an anti-cocaine vaccine display reduced locomotor effects and reinforcing behavior (Kosten and Owens 2005). A study done by Carrera et al. (2001) reported evidence of the efficacy of active and passive immunotherapies for cocaine abuse and evaluated locomotor activity and stereotypic behavior. The active immunotherapy employed a second-generation cocaine immunoconjugate, GND, coupled with keyhole limpet hemocyanin carrier protein (GND-KLH). The passive immunotherapy evaluated a murine anti-cocaine monoclonal antibody comprised of another cocaine immunocongujate (GNC). The mAb used for passive immunotherapy as a mechanistic comparison in this study is referred to as GNC92H2.

A baseline, pre-immunization level of locomotor activity was assessed in 32 male Wistar rats in a 90-minute session after intraperitoneal injection of 15 mg/kg cocaine hydrochloride (HCl). Active immunization of the rats was performed by a bolus injection administered intraperitoneally, containing 250 μg of active protein-drug conjugate in saline at physiological pH. Since this

immunization was active, two booster vaccinations were given at 21 and 35 days from initial immunization to amplify antibody production. Passive immunizations were performed by a bolus injection of 90 mg/kg mAb GNC92H2 in saline administered intravenously, through the tail vein. Animals both actively and passively immunized were subjected to a 15 mg/kg cocaine challenge administered intraperitoneally on the third, seventh, and twelfth day after the last booster (active, GND-KLH) or after initial immunization (passive, GNC92H2), respectively. Both mechanisms of immunotherapy displayed marked decreases in psychomotor effects of cocaine compared to the control. Rats vaccinated with GND-KLH displayed a 76% reduction in locomotor activity compared to baseline upon the first cocaine challenge, and a maximal 80% decrease from baseline in locomotor activity in the last cocaine challenge. Passively immunized rats exposed to GNC92H2 (90 mg/kg) exhibited a 62% decrease from baseline in the first cocaine challenge. Of note, there were also significant differences in stereotyped behavior between groups throughout the 90-minute session, although similar behavioral suppression patterns were seen in both immunization groups. Active immunization with GND-KLH prevented sensitization to cocaine, compared to the control group which displayed increased locomotive and stereotypic behavior as a result of repeated cocaine challenges (Carrera et al. 2001).

Since these findings have been reported, third-generation vaccines using viral gene transfer vectors have been investigated. In a study by Wee et al. (2012) rats vaccinated with a third-generation viral vector anti-cocaine vaccine exhibited decreased motivation to self-administer cocaine when the progressive ratio requirement increased in a dose-response fashion compared to control rats. Also in this study, non-vaccinated animals displayed extinction burst responding when they received saline instead of cocaine upon self-administration, whereas vaccinated animals did not. Cocaine-seeking mechanisms are attenuated or eliminated when antibodies are able to bind to free cocaine molecules and prevent blood brain barrier crossing. One of the more recent cocaine vaccines is TA-CD, which is a cocaine derivative coupled to inactivated cholera toxin B as a functional carrier proteins. The antibodies produced by this vaccine are cocaine-specific, which bind to the drug and break it down utilizing the mechanisms of cholinesterases to fragment the active agent into inactive metabolites for excretion.

Immunotherapy for cocaine addiction has been rapidly developing and has recently made it to clinical trials. A 24-week phase 2b, randomized, double-blind, placebo-controlled study evaluated the efficacy of an active cocaine vaccination in 21 subjects. Vaccinated subjects

produced high levels of IgG anti-cocaine antibodies, which significantly reduced cocaine use in addicts. However, only 38% of subjects were able to maintain high levels of immunoglobulin (Martell et al. 2009). This study was short, had a limited sample size, but offered some interesting data that may contribute to the improvement of anti-cocaine vaccines. First, the quality and efficacy of vaccines and boosters need to be improved to compensate for individual pharmacogenomic differences in antibody production. Third generation vaccines are highly complex, and perhaps future research should focus on the customizability of active immunization. It has been reported in clinical studies that some subjects spontaneously produce low affinity anti-cocaine antibodies, which is likely due to pharmacogenomic variability (Kosten et al. 2014)

As with cocaine, there is no approved pharmacological treatment for methamphetamine addiction (Chen et al. 2013). Methamphetamine is a difficult drug target for immunotherapy since it is such a simple molecule, therefore greatly unnoticeable to the immune system in original form (Julien et al. 2014). In rats, murine mAbs reduce brain exposure when methamphetamine is administered intravenously. Self-administration was also decreased in immunized animals compared to the control, and locomotor activity was greatly reduced (Chen et al. 2013). Three doses of a passive mAb methamphetamine vaccine over a 53-day period produced antibodies in rats, reducing drug distribution to the rat brain by over 60%, but did not attenuate locomotor effects (Kosten and Owens 2005). Until 2012, only passive (mainly mAbs) immunizations for methamphetamine abuse was studied and evaluated by serum concentration and behavioral effects (locomotor activity, self-administration). Preclinical studies in the past five years investigating active immunization with methamphetamine hapten complexes have reported that rats treated with an active vaccine generate high serum-antibody concentrations with high binding affinity (Montoya 2012).

Heroin has been considered an immunopharmacological target since the beginning of this topic in drug treatment research. Bonese et al. (1974) reported decreased motivation to self-administer morphine in rhesus monkeys immunized with morphine hapten conjugated with 6-hemisuccinyl bovine serum albumin (BSA). More recent studies have confirmed that active immunization of an anti-heroin vaccine eliminates self-administration (Stowe et al. 2012) In 2006, Anton and Leff made a significant contribution to the study of drug abuse immunotherapy. They developed a structural formation of a vaccine proven to be bivalent. In other words, the vaccine had cross-reactivity for morphine and heroin. This is important because although immunotherapies

are designed with high specificity, an opiate addict may try to override immunotherapy treatment for oxycodone by using heroin if cravings are strong enough. Bivalent drug vaccines would be an advance in immunotherapeutic technology because it can address a class of drugs rather than just one specific drug. In rats immunized with the bivalent vaccine, significant levels of antibody production with high affinity to morphine and heroin were observed (Anton and Leff 2006). However, although heroin is an important therapeutic drug target, it is challenging to manufacture an effective immunological defense since it has many psychoactive metabolites (Pravetoni et al. 2013).

Other commonly abused opiates have been studied as well. Pravetoni et al. (2013) investigated oxycodone as an immunotherapeutic target. They immunized rats with oxycodone haptens generated with tetraglycine or hemisuccinate linkers conjugated to BSA or KLH; these rats produced high concentrations of antibodies to oxycodone and oxymorphone, an active metabolite, with lower affinities to structurally similar opioids. To evaluate how much oxycodone crossed the blood brain barrier to produce analgesia, this study tested the immunized animals' response to thermal nociception. Immunization against oxycodone significantly reduced oxy-induced analgesia, providing evidence of the attenuation of central effects. This also supports the theory behind immunization against drugs of abuse because by producing antibodies that bind to oxycodone and oxymorphone, less drug is able to reach the brain, therefore analgesic effects are weakened.

Nicotine has also been investigated in active and passive immunization, and has been involved in clinical trials in addition to cocaine vaccines. Nicotine dependence is still highly prevalent, and unlike most other drugs discussed here, it is legal in the United States. Although smoking cessation programs and pharmacotherapies are available, 400,000 deaths occur annually in the United States related to nicotine and smoking (Shorter and Kosten 2011). In rats, a series of active immunization over 4-8 weeks induce antibody production that reduce nicotine distribution to the brain by 40-60% (Kosten and Owens 2005). Importantly, clinically relevant single doses and chronic doses have been studied. Also, it has been reported that in pregnant rats, nicotine administration after immunization decreases fetal brain levels of nicotine, similar to maternal brain levels. It has also been reported that vaccination decreases the ability of nicotine to alleviate withdrawal (Kosten and Owens 2005). This is important because the physical need to avoid withdrawal by compulsively taking drug is a component of addiction – if the drug can no longer

relieve withdrawal, this diminishes the risk of relapse. NicVax and NicOb are vaccines for nicotine abusers that have demonstrated significant results in clinical trials. NicVax has demonstrated a favorable safety profile. In clinical trials, there was no observed increase in compensatory smoking to overcome the effect of the vaccine. The treatment arm receiving the highest dose (200 μg) showed significantly greater abstinence rates 30 days post-study completion (Shorter and Kosten 2011).

Although there are many benefits to advancing the development of immunopharmacotherapy, there are some modifications that need to be made before we can wholeheartedly consider this as a viable treatment option for addiction. In the early 1970's Bonese et al. (1974) developed an early version of heroin immunotherapy. This group reported the effects of an active immunization for heroin treatment, but the specificity of the antibody only recognized heroin, which led to the concern that addicted individuals may just opt for another opiate of abuse during vaccinations. This has been considered when developing second- and third-generation immunotherapies. Another concern is that antibody production in response to active immunization is widely variable among individuals in terms of amount produced and binding affinity to the drug target. This may require further research into the customizability of active and passive immunizations. For active immunization, frequent booster injections are necessary to maintain circulating levels of anti-drug antibodies, typically in a one to six month series of injections. There is a potential issue of compliance here, especially early in treatment when behavioral features of addiction remain largely unchanged. The efficacy of the injection series is also affected by drug-protein conjugate quality, dosage, vaccination frequency, and individual variability in response, i.e. antibody production. Another complication with active immunizations is that it will not be a viable treatment option in addicted individuals with immune deficiencies. This is especially important because human immunodeficiency virus (HIV) is prevalent in drug users that abuse heroin and other drugs administered intravenously. Passive immunotherapy would be the next option, but antibodies generated in another animal and then its immunoglobulins transferred via purified serum still run the risk of transmitting animal viruses and serum sickness. Monoclonal antibodies are usually the preferred option for passive immunotherapies, and they are expensive to produce (Kosten and Owens 2005).

Active and passive immunotherapies against drugs of abuse have shown promising results in animal models of addiction, and clinical trials where applicable. This treatment option is rapidly

developing with an increase in focus for application in acute and chronic overdose (passive immunization only), reduction in drug relapse, and prophylactic uses for at-risk populations (Kosten and Owens 2005). Drug abuse is rampant, with damaging impacts on society and the economy. Ideally, the development of prophylactic immunotherapies for drug abuse should be further studied to reach at-risk youth born to drug-addicted parents or living in urban areas of low socioeconomic status, where drugs are a central and dangerous theme. Perhaps by breaking the cycle of addiction by preventative immunization, illicit drugs would no longer produce reinforcing effects that lead to abuse and addiction, which may be beneficial against fighting the war on drugs, which often seeps into areas of poverty.

There will most likely be future developments in immunotherapy treatment in the direction of advancing adjuvants with greater emphasis on antibody-mediated immunity rather than cell mediated immunity (Alving et al. 2014). The addition of adjuvant agents to basic vaccines may include the introduction of cytokines and other molecules to enhance immune response (Kosten and Owens 2005). Biologic approaches, such as enzyme-based therapies in addition to antibodies and vaccines need to be modified so that individual systems provoke the appropriate immune response with sufficient levels of immunoglobulin production to block drugs (Montoya 2012). Delivery systems may have to be improved as well – perhaps instead of a series of injections for active immunity, a formulation can be created in sustained release form to sustain antibody levels (Kosten and Owens 2005). This development would eliminate the risk of non-compliance in active immunization, since it would be a reformulated single dose. Advances in bivalent vaccines, as described in Anton and Leff (2006), would contribute to the progression of immunotherapy. Immunotherapy is not the end-all, be-all for treating drug addiction although animal models and clinical trials have displayed encouraging data. In fact, if future developments increase this treatment's efficacy for clinical use, it should be an option for drug-addicted patients in combination with behavioral therapy and other necessary pharmacotherapies. Hopefully, this technology will progress and eventually extend to treating addiction to other drugs of abuse not mentioned here, such as ecstasy and other designer drugs.

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