Treatment of methamphetamine abuse: An antibody-based immunotherapy approach

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\section*{ABSTRACT}

Methamphetamine is a highly addictive psychostimulant with tens of millions of abusers around the world. There is no effective or approved medication for treating an addiction to it. Monoclonal antibodies with a high affinity for methamphetamine have the potential to sequester the drug in the vascular compartment and reduce entry into the brain, thereby acting as peripheral pharmacokinetic antagonists without inducing adverse effects on neurons. However, to maintain the antibodies at an effective level, repeated administration is required, which would be expensive and problematic for patient compliance. In this study, we intended to investigate whether using a recombinant adeno-associated virus-mediated gene transfer technique can be an effective approach to achieve long-term expression of anti-methamphetamine monoclonal antibodies in mouse models. We generated a recombinant adeno-associated virus vector encoding the heavy and light chains of an anti-methamphetamine monoclonal antibody, which was constructed in a single open reading frame and linked with a 2A self-processing sequence. In the context of virus-mediated gene transfer, the expression of full-length and functional monoclonal antibodies was successfully demonstrated in vitro and in vivo. Further investigations are ongoing concerning dose optimization, longterm expression, and protection from methamphetamine challenge in mouse models.

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1. Introduction

Methamphetamine (Meth) is an illicit, highly addictive stimulant of the central nervous system (CNS); it is estimated that there are more than 35 million Meth abusers in the world \cite{1}. During the past two decades, governments throughout the world have attempted to conquer Meth abuse; however, it continues to be a major public health problem that needs to be...
tackled on a global scale. Meth abuse can cause numerous adverse biological effects on many organ systems such as acute toxic effects on the cardiovascular system and CNS, acute renal failure, altered behavioral and cognitive functions, and persistent neurological damage to the brain [2,3]. A growing body of evidence has also indicated that Meth can suppress innate and adoptive immunity, and increase susceptibility to viral pathogens such as human immunodeficiency virus [4,5]. There is no approved medication for Meth abuse, and the broad mechanism of Meth action makes it difficult to develop an effective small molecule for pharmacotherapeutic intervention [6,7].

2. The principle of anti-methamphetamine immunotherapy

Antibodies with a high affinity for Meth can sequester the drug molecules in the bloodstream and prevent their gaining access to their activation sites in the CNS or other vulnerable organs (Fig. 1), thereby acting as peripheral pharmacokinetic antagonists that terminate the drug-induced reinforcing effects [8,9]. Because antibodies are too large to readily traverse the blood–brain barrier, an antibody-bound drug molecule cannot freely enter the brain, thus resulting in a drug concentration gradient that allows a free drug molecule to leave the brain at a relatively greater rate than it enters [8].

Fig. 1 – The concept of antibody-based therapy for methamphetamine abuse. Methamphetamine enters the bloodstream, but it can move across the blood–brain barrier (BBB) and freely access its activation sites in the brain, thereby causing adverse psychological and craving effects (left). In the presence of an anti-methamphetamine antibody in the bloodstream, methamphetamine molecules are captured and confined in the circulation, which reduces the entry of the drug into the central nervous system (right). The red closed circles are methamphetamine molecules; the green Y-shaped figures are anti-methamphetamine antibodies.

Fig. 2 – Approaches for generating anti-methamphetamine antibodies in the bloodstream for the treatment of methamphetamine abuse. Antibody-based therapy for methamphetamine (Meth) abuse encompasses three potential approaches: (1) individuals can receive drug-specific vaccines to generate their own anti-Meth polyclonal antibodies; (2) antibodies with a high affinity for Meth can be isolated from antibody libraries there are expressed from various microorganism systems or from antibody-producing hybridoma cells derived from vaccinated animals; these preselected antibodies can be genetically modified to form human-compatible antibodies in suitable expression systems for passive transfer into the peripheral circulation; and (3) expression of anti-Meth antibodies can be achieved by recombinant adeno-associated virus (rAAV)-mediated gene transfer into humans.
3. Approaches for generating anti-methamphetamine antibodies

In general, drug-specific antibodies can be generated in a patient’s bloodstream by active and passive immunization approaches (Fig. 2). In active immunization, drug-like antigens are repeatedly administrated via traditional vaccination procedures to generate a patient’s own polyclonal antibodies against the target drugs [10]. The passive immunization approach is performed by the intravenous administration of well-characterized and genetically engineered monoclonal antidrug antibodies, which are derived from vaccinated animals or antibody libraries [11,12]. In fact, these immunization approaches have been investigated for the treatment of Meth abuse in several rodent models and have shown promising results [13,14].

Drug-specific immunization approaches could potentially avoid inducing unwanted neuromodulatory effects in the brain, although several limitations should be carefully considered prior to treating drug abuse with this approach. The drug molecule itself cannot reactivate the vaccine-induced antidrug immune response to generate antidrug antibodies [15,16]; the preformed antibodies will be disabled by forming long-lived complexes with the captured drugs and be eliminated gradually after being infused into the circulation [10,17].

Therefore, to maintain an effective level of antidrug antibodies, a well-scheduled and repeated administration course is required for both immunization approaches, which would be costly and become a major barrier to patient adherence. In addition, although the drug vaccine is designed to induce drug-specific antibodies, active immunization could potentially induce antibodies with unwanted reactivity to endogenous molecules or tissue antigens, which may then reduce the therapeutic efficacy. Therefore, we proposed an alternative approach for the longterm expression of Meth-specific antibodies in vivo via recombinant adeno-associated virus (rAAV)-mediated gene transfer.

4. Recombinant adeno-associated virus-mediated expression of anti-methamphetamine antibodies

The rAAV vector has been widely used in gene therapy applications, largely because of its low immunogenicity and nonpathogenicity for humans and because of its capability to transduce a broad range of cell types and achieve longterm gene expression [18,19]. In this study, we generated a rAAV serotype-8 (rAAV8) vector carrying an antibody expression cassette (Fig. 3) in which the heavy chain and light chain sequences of a well-characterized anti-Meth monoclonal antibody (K<sub>d</sub> = 11 nM) were separated by a furin-2A self-cleavage site [13,20].

The anti-Meth antibodies expressed in HEK 293 cells infected with the rAAV8 vector were purified from a culture medium, and the antibodies have been shown to capture Meth molecules in a colorimetric assay (Fig. 4). Thus, anti-Meth antibodies expressed from the furin-2A construct are produced in the secreted form and retain the binding activity to Meth molecules. To evaluate rAAV8-mediated expression of anti-Meth antibodies in vivo, adult mice were intraperitoneally injected with a rAAV8 vector (at a dose of 10<sup>9</sup> genome copies/mouse). The serum levels of anti-Meth antibodies were evaluated over time. The anti-Meth antibody was detected at 14 days post-administration of the rAAV8 vector, and a 12-fold higher serum level (p < 0.001) was achieved at 47 days post-administration (Fig. 5A). Longterm evaluation is still ongoing. We further investigated whether anti-Meth antibodies generated by rAAV8-mediated gene transfer can attenuate Meth-induced behavioral changes. Mice were intraperitoneally injected with Meth (1 mg/kg) at 50 days post-administration of the rAAV8 vector. For 90 minutes, the postchallenge locomotor activity was recorded as the total distance traveled. Compared to the locomotor activity of the mock-infected group (n = 4), the Meth-induced locomotor activity in the group (n = 3) receiving the rAAV8 vector was reduced by 30% (p = 0.084; Fig. 5B). The difference did not reach statistical significance; however, these data demonstrated that a single administration of a low dose of rAAV8 is able to achieve peripheral expression of functional anti-Meth antibodies to attenuate Meth-induced behavioral changes in mice. However, this study only presented preliminary results, and the animal numbers were not sufficient to satisfy statistical criteria. The virus vector was also administrated at a low dosage. In future work, there should be numerous chances.

Fig. 3 – Schematic illustration of the genetic construction and biosynthesis of the anti-methamphetamine monoclonal antibody. The heavy-chain and light-chain genes of a preselected anti-methamphetamine antibody are constructed in a single open reading frame, which is linked together by a sequence coding for a combination of furin cleavage site (FCS) and 2A self-processing sequence (derived from the foot-and-mouth disease virus), driven by the cytomegalovirus (CMV) promoter. During protein biosynthesis, the encoded 2A sequence can disrupt peptide-bond formation at the C-terminus of 2A without devastating the synthesis of the downstream protein. The FCS allows enzymatic removal of 2A sequence from the C-terminus of the translated heavy chain. The coexpressed heavy chain and light chain proteins finally self-assemble to form a functional antibody in cells receiving the expression cassette of the anti-methamphetamine antibody. SP = signal peptide.
**Fig. 4** — A colorimetric assay for quantitative determination of anti-methamphetamine antibodies. Antibodies can be captured by protein-G-conjugated sepharose beads. After washing off the unbound methamphetamine (Meth) conjugated with horseradish peroxidase (HRP), the conversion of the HRP substrates to blue-colored substances indicates the presence of anti-Meth antibodies. If color changes do not occur, the captured antibody has no binding activity to Meth. The color intensity is proportional to the amount of anti-Meth antibodies captured by the beads. Anti-V5 is a mouse monoclonal antibody specific to a 14-amino acid V5 epitope derived from the simian parainfluenza virus type 5. The green Y-shaped figures are the antibodies.

**Fig. 5** — The expression of anti-methamphetamine monoclonal antibody in vivo by rAAV8-mediated gene transfer. Male ICR strain mice (8 weeks old age) that were intraperitoneally injected with PBS (n = 4) or rAAV8 (n = 3) carry the expression cassette of an anti-methamphetamine (Meth) antibody. (A) Serum samples were collected at indicated time points (0 days, 14 days postadministration, and 47 days postadministration) for measuring the expression levels of anti-Meth antibodies by colorimetric assay. (B) The animals were challenged with Meth at a dose of 1 mg/kg through intraperitoneal injection on Day 50 postadministration of rAAV8, and the induced locomotor activity was recorded as the total distance traveled (in centimeters) in 90 minutes. All animal studies were conducted under protocols reviewed and approved by the National Health Research Institutional Animal Care and Use Committee. The horizontal lines are the mean distance traveled. Statistical differences are determined by using a two-sample t-test.
and many ways to improve protection from a Meth-challenge following rAAV8-mediated gene transfer.

5. Conclusions

We are conducting further investigations focusing on increasing virus dosages to achieve higher serum levels of anti-Meth antibodies in a sample size with sufficient statistical power. Furthermore, an antibody highly specific to amphetamine (a pharmacologically active metabolite of Meth) is also applied in the rAAV8-mediated gene transfer approach. The safety and efficacy of rAAV-mediated expression of anti-Meth antibodies in humans remains to be addressed. However, we envision that this gene transfer approach, when used in combination with appropriate counseling, would greatly increase the likelihood of successful treatment of Meth dependence. Furthermore, this gene transfer approach could potentially be applied to treatment for other drugs of abuse.

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REFERENCES