Published in final edited form as:

Adv Pharmacol. 2014 ; 69: 107–127. doi:10.1016/B978-0-12-420118-7.00003-2.

Customizing Monoclonal Antibodies for the Treatment of Methamphetamine Abuse: Current and Future Applications

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Abstract

Monoclonal antibody-based medications designed to bind (+)-methamphetamine (METH) with high affinity are among the newest approaches to the treatment of METH abuse, and the associated medical complications. The potential clinical indications for these medications include treatment of overdose, reduction of drug dependence, and protection of vulnerable populations from METH-related complications. Research designed to discover and conduct preclinical and clinical testing of these antibodies suggest a scientific vision for how intact mAb (singular and plural) or small antigen binding fragments of mAb could be engineered to optimize the proteins for specific therapeutic applications. In this review we discuss keys to success in this development process including choosing predictors of specificity, efficacy, duration of action, and safety of the medications in disease models of acute and chronic drug abuse. We consider important aspects of METH-like hapten design and how hapten structural features influence specificity and affinity, with an example of a high-resolution x-ray crystal structure of a high affinity antibody to demonstrate this structural relationship. Additionally, several prototype anti-METH mAb forms such as antigen binding fragments (Fab) and single chain variable fragments (scFv) are under development. Unique, customizable aspects of these fragments are presented with specific possible clinical indications. Finally, we discuss clinical trial progress of the first in kind anti-METH mAb, for which the METH is the disease target instead of vulnerable central nervous system networks of receptors, binding sites and neuronal connections.

Keywords

methamphetamine; monoclonal antibody; drug abuse; preclinical studies; rat; human

Introduction

METH addiction causes serious medical conditions (Table 1). The positive and motivating effects that promote its continued use include a sense of increased energy and euphoria,

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Conflict of Interest Statement

SMO and WBG have financial interests in and serve as Chief Scientific Officer and Chief Medical Officer of IntervXion Therapeutics LLC (Little Rock, AR), a pharmaceutical biotechnology company focused on treating human drug addiction with antibody-based therapy.

heightened awareness, self-confidence, increased sexual drive and performance, and reduced appetite (Cho, 1990; Rawson, Washton, Domier, & Reiber, 2002). Unfortunately, these initial positive effects are reinforcers of METH use and likely play a role in the establishment of METH addiction. When METH users attempt to continue the pleasurable effects through more frequent use, tolerance develops (Cho, 1990; Cook et al., 1993). To maintain their “high”, users often increase the METH dose and shorten the interval between self-administrations. They accomplish this with a so-called “speed run”, which usually includes rapid input of the drug by smoking, snorting, or intravenous (i.v.) delivery. While initial doses of 10 mg of METH are common, 150 mg to 1 g doses are sometimes consumed during a binge period (Cho, 1990). Methamphetamine is chief among a class of dangerous stimulants that include amphetamine (AMP, also a metabolite of METH), and the structurally related stimulant (+/-) 3,4-methylenedioxymethamphetamine (MDMA, or ecstasy) (Figure 1, note MDMA is shown as the (+)-isomer and not as a racemic mixture).

Binge use may lead to adverse complications that require medical intervention. As indicated by the most recent reports from 2011, METH/AMP-related problems accounted for 159,840 emergency department visits, which was 12.8% of the visits resulting from all illicit drugs (DAWN, 2013). This represents a 71% increase over 2009 statistics. When METH users come to emergency departments, many need treatment for acute toxicity or psychiatric symptoms. The number of METH/AMP users that sought chronic detoxification and longer-term treatment for their drug use increased nearly 50% between 2009 and 2011.

When METH is used at high binge doses the medical effects can be very prolonged, especially since METH's half-life is about 12 hr in humans (Cook et al., 1993; 1982; Mendelson et al., 2006). To recover, users might sleep for days (Kramer, 1967). This abusive cycle of METH use causes severe depression and sometimes a psychosis that is similar to paranoid schizophrenia (Derlet & Heischouer, 1990a; Lee, Boehm, Chester, Begent, & Perkins, 2002). Abusive and violent behaviors often occur (Kramer, 1967). Serious or medically harmful effects are so significant at higher METH doses (see Table 1) that studies of these effects are not safe in humans, thus alternative animal models are required.

METH Mechanisms of Action, Toxicity, and Current Therapies

The actions of METH in the central nervous system (CNS) result from complex interactions with a diverse set of neurotransmitters, ion channels, and presynaptic catecholamine uptake systems (Seger, 2010). The effects on the CNS dopaminergic system are particularly profound because METH is a substrate for the dopamine (DA) transporter, leading to extraneural DA transport. METH can also increase neuronal release of DA through processes not controlled by the DA transporter. Together, these increased levels of presynaptic DA cause increased post-synaptic transmission (Cho, 1990). Other important METH-induced brain changes lead to excitation, excessive motor movements, mood changes, and a suppressed appetite. The CNS clinical syndromes resulting from METH's diverse mechanisms at multiple CNS sites emphasize the difficulty in discovering a single small-molecule agonist or antagonist that could safely treat METH effects without addiction liability.

Effective medications are needed for the treatment of the plethora of medical problems related to METH abuse, which range from drug overdose to long-term neurotoxicity. Current therapies for METH medical effects are largely supportive. For instance, in overdose situations, patients are treated with hydration, anticonvulsants/sedatives, antihypertensives, or surface cooling while medical personnel wait for the most severe effects to dissipate (Beebe & Walley, 1995; Derlet & Heischouer, 1990b). While behavioral therapies for METH abuse exist, effective pharmacotherapies for drug abuse and addiction are missing. According to the National Institute on Drug Abuse (NIDA), the optimal characteristics of an effective addiction treatment program would combine counseling and therapeutic medicines, along with support services to meet individual patient needs.

Therapeutic Monoclonal Antibodies to Counteract METH Effects

Immunopharmacological treatments fall into one of two broad categories (Gentry, Rüedi-Bettschen, & Owens, 2009; Kosten & Owens, 2005). The first is active immunization, which requires 2-3 months of immunizations with a drug-protein conjugate before a long-lasting immunological memory against the drug of abuse is established. This therapeutic strategy will not be discussed in this review. The second potential treatment is administration of mAb (singular and plural). This involves the infusion of a high quality, pharmaceutical grade antibody for immediate and long-term protection against the drug of abuse, without creating an immunological memory. The mAb dose and frequency of dosing can be easily controlled, unlike the variable patient-to-patient response sometimes active immunizations (Roskos, Davis, & Schwab, 2004). In addition, the beneficial response from infusion of a mAb can be immediate and long lasting. This makes it feasible to treat both the immediate urgency of a drug overdose, as well as chronic drug addiction. The major disadvantages are cost of the medication and the possibility of allergic reaction to the protein.

Therapeutic mAb represent a rapidly growing area of clinical drug development, with more than 30 mAb medications already approved by the US Food and Drug Administration. These therapeutic mAb have diverse mechanisms of action including: 1) neutralizing toxins or drugs, 2) delivering drugs to targeted sites, and 3) altering cellular function (Lobo, Hansen, & Balthasar, 2004). The first of these mechanisms best describes the mode of action for mAb treatment of drug abuse. To summarize their actions, anti-METH mAb changes both the amount and the rate of METH entry into medically critical site(s) of action in the CNS and cardiovascular system (Gentry, Rüedi-Bettschen, & Owens, 2010).

Three important and unique properties of mAb are the long duration of action of IgG mAb, the disease target for the mAb medications is the METH molecule, and the mAb medications modulate METH effect without leaving the blood stream. This medication profile is profoundly different from most small molecule agonist and antagonist that are short acting, target the binding sites within the CNS, and must cross the blood brain barrier to be effective (Camí & Farré, 2003).

Table 2 summarizes some of the most important prototype antibodies and antibody binding fragments that could be used for treating medical problems caused by METH abuse. MAb

medications are classified as pharmacokinetic antagonists because they bind to the target molecule (METH) and alter its distribution, metabolism, and elimination. There is no abuse liability, which is a negative characteristic of many small-molecule agonist therapies. Because the mAb rapidly binds METH in the blood stream and prevents it from reaching its sites of action, the mAb or antibody fragment can reverse or prevent acute toxicities (Gentry et al., 2010; Hardin, Wessinger, Proksch, & Owens, 1998). The mAb dose and frequency of dosing can be easily controlled to achieve the desired level of effect, unlike active immunization. Finally, mAb have a long elimination half-life (~7 days in rats and ~21 days in humans; (Bazin-Redureau, Renard, & Scherrmann, 1997; Joos et al., 2006), which limits the need for frequent repetitive dosing and improves the likelihood of patient compliance. This makes IgG mAb a very novel prototype for treating the chronic effects of addiction.

Predicting Therapeutic Effectiveness

The complex pharmacokinetic relationship between mAb and a target ligand(s) makes it difficult to predict therapeutic effectiveness without an integrated program of mAb design and preclinical testing. Optimizing the mAb medications requires a multidisciplinary approach including chemistry, immunology, behavioral science and clinical pharmacology. Important factors mediating mAb efficacy include mAb affinity and specificity, molar ratio of the mAb:drug, time of mAb administration in relation to METH use, dose of mAb, and disposition of mAb *in vivo* (Lobo et al., 2004; Peterson, Laurenzana, Atchley, Hendrickson, & Owens, 2008).

While the focus of this review is not on behavioral models of addiction, we use several important behavioral models to discover the best anti-METH mAb for treating METH abuse. These include measures of changes in locomotor activity (Byrnes-Blake et al., 2003; Gentry et al., 2006), mAb effects on drug discrimination (McMillan, Hardwick, Li, & Owens, 2002), on METH self-administration (McMillan et al., 2004), and on the cardiovascular system (Gentry et al., 2006). While it is essential to use these types of preclinical testing, there are no rodent models that are proven to predict human clinical efficacy.

METH Metabolism and Pharmacokinetics-Choosing the Appropriate Animal Testing Model

In addition to understanding how these antibody-related factors contribute to the pharmacokinetic mechanisms of mAb effects, it is also important to understand how METH metabolism and pharmacokinetics impact mAb effects. Because AMP is a major psychoactive metabolite of METH, it is necessary to consider the pharmacokinetic properties of METH and AMP in humans and how they relate to the values in rats (our primary preclinical animal model). The METH pharmacokinetic values for the male rat (Rivière, Byrnes, Gentry, & Owens, 1999) and man (Cook et al., 1993) after i.v. administration are: volume of distribution (Vd), 9.0 L/kg vs. 3.7 L/kg; systemic clearance (Cl), 126 ml/min/kg vs. 3.2 ml/min/kg; and terminal elimination half life ($t_{1/2\lambda_z}$), 63 min vs. 13.1 h, respectively. While the pharmacokinetic values for Vd for the two species differs only by a factor of 2.4, the systemic clearance (Cl) is 39-fold greater in the rat. Metabolism

of METH is the major route of elimination in the rat, with renal elimination constituting only a minor route of the total clearance (9-13% of the dose). In contrast, renal elimination is a significant component of human CIs, with 37-45% of the METH dose appearing in the urine (Cook et al., 1993). These data suggest physiologic and treatment factors that could increase urinary elimination of METH could be an effective treatment in humans. A possible candidate for this therapeutic strategy is anti-METH antigen binding fragment or single chain antibodies (Fab or scFv, respectively, Table 2), which are primarily cleared by kidney passive filtration. For example, the use of a monoclonal anti-PCP Fab can significantly increase renal passive filtration of PCP in rats (Proksch, Gentry, & Owens, 1998). Although an anti-METH scFv can rapidly change the apparent volume of distribution of METH in serum, the scFv's specific effects on METH clearance by individual organs like the kidney and liver has not been determined (Peterson et al., 2008).

The short $t_{1/2\lambda z}$ of METH in rats (about 1 h), compared with that in humans (about 13 h), appears mostly due to a significantly greater capacity for metabolic elimination in the male rat (Milesi-Hallé, Hendrickson, Laurenzana, Gentry, & Owens, 2005). Because METH is partially cleared by the CYP2D6 enzymatic pathway in humans (Lin et al., 1997), and approximate 5-10% of the Caucasian North American population are deficient in this enzymatic pathway (Kimura, Umeno, Skoda, Meyer, & Gonzalez, 1989), many METH users will be poor metabolizers of the drug. These patients may be more profoundly affected for longer periods of time and more vulnerable to adverse effects.

Because of some limitations with the male rat as an animal model of human METH use, we explored the possible advantages of using the female rat as a preclinical model (Milesi-Hallé et al., 2005). We find sex-dependent differences in METH pharmacokinetics, metabolism, and locomotor response between male and female rats, with females (but not males) showing dose-dependency in the pharmacokinetic parameters at the highest dose tested (3 mg/kg). Dose-dependent METH (Cook et al., 1993) and MDMA (la Torre et al., 2000) pharmacokinetics are reported in male humans. There are no reports in females. We also find the female rat has a significantly slower METH CIs and non-renal clearance, lower AMP formation, and greater METH urinary excretion compared to the male rat. Based these parameters, the female rat pharmacokinetic profile is more consistent with METH pharmacokinetics in male humans. However, the differences are not great enough to prefer the use of female to the male rat. Rather, we view the female and male rats as representative models of human patients with different levels of METH vulnerability.

The Role of Affinity, Binding Capacity and *In Vivo* Modifications in mAb Function

Optimizing and maximizing *in vivo* affinity of the mAb for METH is a critical first step (Byrnes-Blake, Laurenzana, Landes, Gentry, & Owens, 2005; Peterson et al., 2007). For instance high-affinity anti-METH mAb6H4 ($K_d = 4$ nM) is much more effective at antagonizing METH locomotor effects than the lower affinity anti-METH mAb6H8 ($K_d = 250$ nM). Other studies from our laboratory with an anti-phencyclidine mAb (anti-PCP mAb6B5, $K_d = 1$ nM) show that binding capacity for the PCP does not limit therapeutic effectiveness as much as was initially predicted based solely on number of binding sites

(Hardin et al., 1998; Laurenzana, Gunnell, Gentry, & Owens, 2003b; Proksch, Gentry, & Owens, 2000). In these studies, a mAb dose of only 1/100th the PCP body burden significantly decreased brain PCP concentrations, reversed PCP-induced behavioral effects, prevented adverse PCP-induced health effects, and even prevented death.

We think the mechanism of these favorable effects results from synergy among several following factors; (1) the blood–brain barrier restricts the antibody to the plasma in the blood stream of the brain but METH freely crosses the blood-brain barrier, (2) there is an extremely small volume of the vascular cerebral space relative to the general circulation, and (3) the amount of METH in the brain is an extremely small fraction of the total METH dose (0.1% of the total dose) (Laurenzana, Byrnes-Blake, Milesi-Hallé, Gentry, Williams, et al., 2003a). These factors, along with the mAb's high affinity and rapid association with the PCP, act synergistically to allow temporary greater drug-mAb occupancy with each pass through the brain. This is an important discovery indicating that mAb pharmacokinetic antagonism of PCP (and perhaps METH) actions might not be as easily “surmounted” as previously thought.

In vivo changes that alter mAb affinity, binding capacity, or pharmacokinetics have an impact on mAb effectiveness. Studies from our laboratory indicate that our high-affinity anti-METH mAb6H4 may be at least partially inactivated *in vivo* (Byrnes-Blake et al., 2003). Although anti-METH mAb6H4 protected against METH-induced locomotor activity acutely over the first several hours, the effectiveness is reduced for METH challenges given three days later and significantly diminished after seven days. This contrasts with other studies showing a single dose of anti-PCP mAb6B5 protects against PCP-induced effects for at least two weeks (Hardin et al., 2002; Laurenzana, Gunnell, Gentry, & Owens, 2003b) and can still reduce PCP brain concentrations one month later in rats (Proksch et al., 2000).

The greatly reduced effectiveness of our anti-METH mAb6H4 after 24 h suggest that the mAb is somehow altered *in vivo* to affect affinity and/or binding capacity. The experimental data shows there are no changes in the pharmacokinetic properties, such as increased clearance (Laurenzana et al., 2009). Thus, binding function, such as changes in K_d or capacity appears to be the reason for the unexpected loss of the mAb6H4 binding function. Fortunately a redesign of the METH-like hapten solved the problem (Carroll et al., 2009; Owens, Atchley, Hambuchen, Peterson, & Gentry, 2011).

Hapten Design for Improved Target Specificity and mAb Functionality

The hapten design and IgG DNA sequencing described in this section has been ongoing in our laboratory group over the past decade (Carroll et al., 2009; 2011; Peterson et al., 2007; Peterson, Owens, & Henry, 2006). Through experience in molecular modeling and sequence analysis, we were able to analyze a library of mAb function and sequence data, and then elucidate important IgG sequence and structural features involved in antibody-METH interactions. This work revealed key hapten features that are important for shaping critical molecular interactions during immunization (Peterson et al., 2006).

Because the chemical composition and molecular orientation of the drug-like haptens on the antigen are crucial determinants in generating antibodies against small molecules

(Landsteiner & Van der Scheer, 1931), the goal of these long-term studies was to discover design criteria needed to produce long-acting anti-METH, anti-AMP and anti-MDMA mAb medications. This led to discovery of mAb medications with the potential specificity needed to treat overdose and addiction indications resulting from METH, AMP and/or MDMA abuse. Although high affinity for METH was always our foremost goal, we wanted to generate a single mAb with high affinity for the metabolite (+)-AMP and a related compound, (+)-MDMA. This is the (+)-isomer in the racemic mixture known as MDMA or ecstasy.

Molecular orientation of the hapten on the protein carrier is critical to the control of antibody specificity for its target ligand. This is because antibodies generated against hapten-protein conjugates primarily react to the portion of the hapten antigenic epitope that is distal to the point of chemical attachment on the carrier protein. Our goal was to generate antibodies against the (+)-isomers of METH-like compounds. (+)-Isomers produce significantly more psychomimetic effects, locomotor activity, stereotyped behavior, monoamine oxidase inhibition, and addiction liability than (–)-isomers (Cho, 1990; Marquardt, DiStefano, & Ling, 1978). From a chemical synthesis point of view this meant the hapten linker needed to be attached to the phenyl ring structure of (+)-METH, to increase the distance from the chiral center. This maximized the immune systems potential to select for antibodies with high affinity and specificity for (+)-isomers (Peterson et al., 2007).

Using these criteria, we progressively generated haptens with changes in linker length (*e.g.*, 4, 6, and 10 molecules), molecular modification to the linker constituents (*e.g.*, addition of an oxygen molecule to mimic the oxygen in MDMA), and attachment to the METH phenyl ring at the para, ortho and meta positions. The longer (+)-METH MO10 spacer was created to allow more flexibility of the hapten when attached to the carrier protein, in hopes of discovering mAb(s) with broader recognition of (+)-METH-like structures (Carroll et al., 2009; Peterson et al., 2007). Using these haptens, we generated groups of antibodies with different affinities for METH (from 4-250 nM) and specificities (METH, AMP, (+)-MDMA). While we hypothesized that mAb affinity is the primary driving force for therapeutic efficacy, we learned that the duration of action and function of our anti-(+)-METH mAb *in vivo* cannot be predicted from simple *in vitro* characterization or from the pharmacokinetic properties of the antibodies (Laurenzana et al., 2009), as discussed in an earlier section.

Our first generation of mAbs was produced from short chain haptens linked to the phenyl ring structure of METH. The linker chains had 4 and 6 molecule spacer groups bound at the para and meta positions of the phenyl ring of the hapten. The other end of the spacer was designed for easy covalent attachment to amino acids of the protein carrier (Peterson et al., 2007). The resulting mAbs had virtually no cross reactivity with AMP, but high affinity for METH and (+)-MDMA. When we produced our second generation of haptens with a 10 molecule spacer and oxygen attached directly to the METH component of the hapten, we began to discover mAb with specificity for METH, AMP and (+)-MDMA. One mAb in particular (mAb4G9) had the additional advantages of increased duration of action of METH binding *in vivo* and improved efficacy (Carroll et al., 2009; Owens et al., 2011). MAb4G9 also had a very long duration of *in vivo* function (Laurenzana et al., 2009). We

now know this is related to important chemical properties of the MO10 hapten, since we have recently identified other long acting mAb from immunization with MO10 hapten. One of these mAb is mAb7F9 (METH K_d = 9 nM), which is our lead candidate medication and is currently in Phase I clinical trials for treating METH abuse.

Relationship between Hapten Structure and mAb Function; Application to Other Antibody Forms

Numerous studies have attempted to elucidate structural features of antibodies that lead to high affinity and specificity binding. The structural classifications of antibody binding sites for ligands are generally grouped into four classes: concave, moderately concave, rigid, and planar (MacCallum, Martin, & Thornton, 1996). The crucial interactions for mAb binding are mediated primarily by the amino acids within the complementarity determining regions (CDR) of the antibody heavy and light chains. CDR contacts for domains on protein antigens generally involve the side chains of 15-20 amino acids (Poljak, 1973). Very small molecule drugs like METH (149 Da) interact with a limited number of these amino acids due to the small surface area and low number of charged atoms. This likely limits the potential for discovery of extremely high affinity mAb binding (*e.g.*, 200 pM), and is a major factor in why even our highest affinity antibodies are only in the low nanomolar range of K_d values (*i.e.*, 4-9 nM).

Information derived from X-ray crystallography studies help to elucidate antibody mechanisms of binding, and provide the foundation for customizing affinity and specificity. We first determined the crystal structures of a Fab derived from murine anti-PCP mAb6B5 (Lim, Owens, Arnold, Sacchettini, & Linthicum, 1998) and later the structure of Fab fragments from anti-METH mAb (Celikel, Peterson, Owens, & Varughese, 2009) in complex with their target drugs of abuse. Both of these antibodies have a deep pocket mode of binding (as demonstrated for METH in Figure 2). A unique feature of the binding site of anti-PCP mAb6B5 is a tryptophan at position 97H (H-chain). The side chain of this residue acts as a hydrophobic umbrella over the ligand in the antigen binding pocket, which appears to significantly contribute to its high affinity for PCP (Lim et al., 1998).

Since IgG molecules of anti-METH mAb are difficult to crystallize due to their flexible nature, antibody structure is studied by examinations of other, smaller customized forms (Table 2). Specifically we use the antigen binding fragments Fab and scFv. Anti-METH ScFv6H4 binds METH with high affinity and the crystal structure of this complex shows that the METH molecule is encased almost completely in a deep binding pocket. In fact, only 3% of the surface area of METH is solvent accessible in the structure of the complex (Figure 2). The METH ligand is oriented in the binding site with the aromatic ring towards the entrance and the side chain pointing to the interior. This is not surprising since the hapten used to discover mAb6H4 contains a linkage of the aromatic ring to the para position of the METH aromatic ring structure (Peterson et al., 2007).

Both hydrophobic interactions and hydrophilic interactions appear to stabilize METH binding. The entrance of the binding site is lined with six aromatic residues. These combine to form a hydrophobic barrel around the aromatic portion of METH that covers 68% of the

METH surface area. This forms the majority of interaction surface for the METH:scFv6H4 configuration. Although the binding is mediated mostly by hydrophobic interactions, the secondary amine of METH plays an important role because it is the only charged atom in METH (as well as AMP and MDMA). A salt bridge exists between this nitrogen and the carboxyl oxygen of a key glutamate from the heavy chain and a second hydrogen bond to a histidine of the light chain. Thus it appears that the para-position orientation of the original METH-like hapten linker favored the formation of this crucial hydrogen bond. If the linker were at another position, the charged nitrogen might not be exposed and available for interaction with amino acid residues so deep in the pocket. While this binding configuration generated very high affinity anti-METH antibodies, this mAb proved ineffective for chronic treatment with the intact mAb6H4. Fortunately, other high affinity antibodies against METH have been discovered, which are also long acting *in vivo* (Owens et al., 2011).

Antibody Form and Duration of Action

With a half-life of about three weeks (Joos et al., 2006), IgG isotype mAb exhibit one of the longest durations of action of any small molecule or biological-based medications. The long half-life (150 kDa) is due in part to the ability of the antibody to be repeatedly salvaged from catabolism pathways by the neonatal Fc receptor (FcRn) through interactions with the FC portion of the IgG (Lobo et al., 2004; Roskos et al., 2004). Under normal conditions, IgG circulates in the vasculature where it is internalized via pinocytosis into epithelial cells. In the next step of the process, IgG is recycled in a pH-dependent manner (Ghetie & Ward, 2002). If this recycling occurs, the antibody is protected from degradation and released back into the vasculature. While FcRn-mediated recycling of IgG dramatically increases the mAb half-life in the circulation, the process exposes the protein to microenvironment pH changes. This can potentially alter the protonation states of amino acid side chains and affect the binding pocket charge and protein conformation (Sinha, Mohan, Lipschultz, & Smith-Gill, 2002).

Small antibody binding fragments such as Fab and scFv lack the IgG domains required for FcRn binding, thus are not subject to recycling and the associated pH-dependent change. Their clearance is predominately dependent on renal elimination (Proksch et al., 1998). Thus their half-life is much shorter. In clinical situation like METH overdose or other acute treatment scenarios, this short half-life could be advantageous. For instance, a short-acting Fab or scFv could be used to rapidly clear the body of high doses of METH and other stimulants (Peterson & Owens, 2009). Treatment of rats with an anti-METH scFv6H4 monomer shows the scFv6H4 monomer is quickly converted to multivalent forms or cleared. This results in an apparent $t_{1/2\lambda n}$ of 5.8 min (Peterson et al., 2008). In contrast, the larger scFv6H4 multivalent forms (*e.g.*, trimers) that spontaneously associate *in vivo* show a $t_{1/2\lambda n}$ of 3.8 hrs. ScFv6H4 binding to METH directly correlates with scFv6H4 serum molar concentrations. These data suggest the scFv6H4 binding sites are (nearly) fully occupied in the serum and that high affinity METH binding is the cause of the significant increases in METH serum concentrations.

These data also suggest that the scFv6H4 multimers (and not the monomer) are responsible for the increase in duration of action. *In vitro* multimer formation is often seen in scFv

proteins; the relative amounts of each form depend upon linker length, pH, ionic strength, and presence or absence of antigen (Arndt, Müller, & Pluckthun, 1998). This dimerization between the V_L and the V_H domains can occur through two proposed mechanisms (Hudson & Souriau, 2003). In the first scenario, the domains “open” or separate around the linker and bind to the domains of another unhinged scFv. This scenario results in an intermediate that is unable to bind to antigen since the binding site requires the coordination of both domains. The second mechanism is a simpler “back to back” conformation. In this scenario the regions of the V_L and the V_H domains associate with those of another scFv, instead of forming interactions with globular domains of the constant region. This conformation does not require temporary loss of activity and we think this is the mechanism through which anti-METH scFv6H4 form multimers. Especially since it is able to bind to METH in equimolar ratios during the entire time in the serum.

Because multimerization can extend half-life, it has been explored as a method to customize the pharmacokinetic properties of antibodies. Unfortunately, because multimerization is not always predictable, it is unlikely that modification of the scFv solely through molecular engineering would produce reliable products. The main weakness in this approach is that the scFv molecules tend to self-associate in unpredictable mixtures of dimers and trimers, and larger molecular weight multimers (Dolezal et al., 2000). This leads to large-scale production problems and less than optimal formulations.

While scFv has potential advantages over IgG in terms of cost, ease of production, and amount of protein needed for treatment, the variability of scFv multimer formation needs to be addressed. Fortunately, the conversion of the IgG to the single gene scFv format makes molecular re-engineering easier. For instance, the cysteine codon can be incorporated into the DNA coding sequence as a potential conjugation site on the protein for addition of polyethylene glycol (Chapman, 2002; Lobo et al., 2004) or nanoparticles (Nanaware-Kharade, Gonzalez, Lay, Hendrickson, & Peterson, 2012). These customizable fragments present many opportunities for tailoring individual therapies for specific clinical indications in the next generation of immunotherapies for treatment of drug abuse.

Conclusions and Future Directions

From our anti-METH mAb discovery process we have chosen the murine anti-METH mAb7F9 (Carroll et al., 2009; Owens et al., 2011) to develop as a human treatment for METH addiction. MAb7F9 was converted to a mouse-human chimeric antibody by reengineering the protein to include the METH-binding variable regions from the original murine antibody (i.e., mAb7F9, $K_d = 9$ nM), and the constant domains from a human IgG2 κ mAb. This form of the antibody has been administered to humans as part of a Phase 1 clinical trial. Preliminary analysis of the data from this study suggests it will be safe for administration to humans. In the future, we think it will be possible to further customize our anti-METH mAbs to significantly improve on the affinity constants, or to shorten or prolong the half-life of the antibodies to improve their utility for treatment of overdose and detoxification.

Acknowledgments

The National Institute on Drug Abuse (DA11560, DA026423, DA031944), Arkansas Biosciences Institute (the major research component of the Arkansas Tobacco Settlement Proceeds Act of 2000), and the National Center for Advancing Translational Sciences (UL1TR000039) supported this work.

Abbreviations

AMP	(+)-amphetamine
CDR	complementarity determining region
Cl_s	systemic clearance
CNS	central nervous system
DA	dopamine
Fab	antigen binding fragment
FcRn	neonatal Fc receptor
IgG	immunoglobulin gamma
i.v.	intravenous
K_d	equilibrium dissociation rate constant
LC-MS/MS	liquid chromatography coupled with tandem mass spectrometry
mAb	monoclonal antibody
(+/-)-MDMA	(+/-) 3,4-methylenedioxymethamphetamine
METH	(+)-methamphetamine
PCKN	pharmacokinetics
PCP	phencyclidine
scFv	single chain variable fragment
t_{1/2λn}	terminal elimination half-life
V_d	apparent volume of distribution
V_H	variable heavy chain
V_L	variable light chain

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Figure 1. Chemical structures of (+)-Methamphetamine, (+)-Amphetamine, and (+)-3,4-methylenedioxymethamphetamine

Figure 2. X-ray crystal structure of a high affinity anti-METH scFv6H4 reveals METH binding configuration is influenced by hapten design

Top (A) and side (B) views of anti-METH scFv6H4 crystal structure showing that METH is bound in a deep cavity, which tightly fits the shape of METH. The position of the para-carbon of the METH aromatic ring, the attachment point of the 6-carbon linker of the hapten, is nearest the entrance to the binding pocket. This structure strongly supports that the binding pocket shaping is dictated by hapten design. Binding pocket surface representations are colored by binding property. Molecular interactions are a balance of neutral (white), hydrophobic (green), hydrogen bonding acceptor (red) and hydrogen bonding donor (blue) potentials. Two water molecules in the pocket also form key hydrogen bonds with METH (not shown for clarity).

Table 1Acute and Chronic Medical Problems Caused by Methamphetamine Abuse. ^a

Acute Medical Problems ^b	
Neuropsychiatric	Aggression, acute psychosis, violence
CNS	Cerebral hemorrhage, hyperthermia, stroke and seizures leading to brain injury
Cardiovascular	Dysrhythmias, hypertension, myocardial infarction, vasoconstriction
Renal	Acute renal failure caused by rhabdomyolysis
General Health	Death

Chronic Medical Problems ^c	
Neuropsychiatric	Addiction, delirium, paranoia, hallucinations, psychosis
CNS	Neurological changes including short-term memory loss, neurotoxicity, cerebral hemorrhage, hyperthermia, stroke and seizures
Cardiovascular	Dysrhythmias, hypertension, myocardial infarction
Hepatic	Liver toxicity
Renal	Electrolyte imbalance
General Health	Malnutrition, significant weight loss, increased risk of infectious diseases (i.e., hepatitis, HIV/AIDS)

^a These medical effects of METH abuse are adapted from the report of (Gentry et al., 2009) and compiled from the following references.

^b Acute effects: (Albertson, Derlet, & Van Hoozen, 1999; Callaway & Clark, 1994; Mendelson, Jones, Upton, & Jacob, 1995; Richards et al., 1999)

^c Chronic effects: (Albertson et al., 1999; Hong, Matsuyama, & Nur, 1991; Nordahl, Salo, & Leamon, 2003; Richards et al., 1999; Sato, 1992; Urbina & Jones, 2004; Yu, Larson, & Watson, 2003)

Table 2

Anti-METH mAb forms designed for customization of duration of action and specific clinical treatment scenarios.

mAb Medication (molecular size) $t_{1/2\alpha n}$	Possible human medical indication	Immunochemical selection criteria	Pharmacological testing and expected mAb-induced changes in rats
<p>IgG (150,000 Da) Weeks</p>	<ul style="list-style-type: none"> • Overdose • Detoxification • Reduce harmful health effects from binge use 	<ul style="list-style-type: none"> • Kd for METH <20 nM • Kd for (+)-AMP and/or and (+)-MDMA of <20 nM • No significant cross-reactivity with other medications or neurotransmitters 	<ul style="list-style-type: none"> • Favorable pharmacokinetic profile • Long-acting METH binding in rodents (i.e., minimum of 2 weeks) • Reduction of METH-induced locomotor activity • Reduction of adverse medical effects
<p>Fab (50,000 Da) Hours</p>	<ul style="list-style-type: none"> • Overdose • Detoxification 	<p>Above plus:</p> <ul style="list-style-type: none"> • Ease of production • Lower protein dose required for drug binding 	<ul style="list-style-type: none"> • Short-acting METH binding in rodents (i.e., 8 hrs) • Short-term reduction of adverse medical effects
<p>scFv (25,000 Da) Minutes to hours</p>	<ul style="list-style-type: none"> • Overdose • Detoxification 	<p>Above plus:</p> <ul style="list-style-type: none"> • Easily modified through molecular engineering • Can be modified to improve duration of action or function (e.g., Pegylation, nanoparticles) 	<ul style="list-style-type: none"> • Very short-acting METH binding in rodents (i.e., 2-3 hrs) • Short-term reduction of adverse medical effects