Pharmacological Effects of a Monoclonal Antibody against 6-Monoacetylmorphine upon Heroin-Induced Locomotor Activity and Pharmacokinetics in Mice

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ABSTRACT

Immunotherapy can provide a supplemental treatment strategy against heroin use on the principle of sequestering the active drug in the bloodstream, thereby reducing its distribution to the brain. Previous studies have shown that heroin’s first metabolite, 6-monoacetylmorphine (6-MAM), is the main mediator of acute heroin effects. The objective of the present study was to characterize the pharmacological potential of a monoclonal antibody against 6-MAM (anti-6-MAM mAb) to counteract the heroin response. The individual contributions from heroin and 6-MAM to heroin effects were also examined by pretreating mice with anti-6-MAM mAb (10–100 mg/kg) prior to either heroin or 6-MAM injection (1.25–2.5 μmol/kg). The opioid-induced behavioral response was assessed in a locomotor activity test, followed by opioid and antibody quantification in blood and brain tissue. Pretreatment with mAb caused a profound reduction of heroin- and 6-MAM-induced behavior, accompanied by correspondingly decreased levels of 6-MAM in brain tissue. mAb pretreatment was more efficient against 6-MAM injection than against heroin, leading to an almost complete blockade of 6-MAM-induced effects. mAb pretreatment was unable to block the immediate (5-minute) transport of active metabolites across the blood-brain barrier after heroin injection, indicating that heroin itself appears to enhance the immediate delivery of 6-MAM to the brain. The current study provides additional evidence that 6-MAM sequestration is crucial for counteracting the acute heroin response, and demonstrates the pharmacological potential of immunotherapy against heroin use.

Introduction

Heroin is known for its potent reinforcing effects and is one of the most widely used addictive drugs worldwide (UNODC, 2015). Standard treatment of heroin addiction includes opioid substitution therapy with agonists such as methadone and buprenorphine, which sustain addiction by mimicking the rewarding actions of heroin. Agonist treatment also contributes to overdoses and may have cognitive side effects (Verdejo et al., 2005; Andersen et al., 2011; Bernard et al., 2015). Antagonist treatments include naloxone and naltrexone, which act by blocking the action of opioid drugs and may potentially interfere with the action of endogenous opioids (e.g., endorphins and enkephalins) (Nestler, 2002; Bodnar, 2016; Wardle et al., 2016).

Immunotherapy can provide an alternative or additive strategy for inhibiting opioid effects on the principle of sequestering the drug in the bloodstream and thereby reducing its distribution to the brain (Shen and Kosten, 2011). Immunotherapy comprises active vaccination, in which the immune system is triggered to endogenously produce drug-specific antibodies, or passive immunization, in which preformed antibodies are administered directly by injection (Peterson and Owens, 2009; Shen and Kosten, 2011). Whereas recent studies have used an active vaccine strategy against opioids (Stowe et al., 2011; Pravetoni et al., 2012; Raleigh et al., 2013, 2014; Schlosburg et al., 2013), the potential for monoclonal antibodies (mAbs) against heroin-induced effects has received minor attention (Killian et al., 1978; Bogen et al., 2014).

A challenge with immunotherapy against heroin is the rapid transformation of heroin into several active metabolites, which are capable of traversing the blood-brain barrier (BBB) and binding to opioid receptors (Selley et al., 2001; Rook et al., 2006). With an in vivo half-life of 2.5 minutes in mice and between 1.5 and 8 minutes in humans, heroin is quickly deacetylated into 6-monoacetylmorphine (6-MAM) (Way et al., 1960; Rook et al., 2006). After subcutaneous injection in mice, heroin reaches its peak blood concentration within 1 minute, whereas the maximum 6-MAM concentration is reached after 5–10 minutes. Although not well established, previous studies have indicated an in vivo half-life of 10–15 minutes for 6-MAM in mice (Andersen et al., 2009; Boix et al., 2013). 6-MAM is...
further metabolized to morphine, which is ultimately glucuronidated into the pharmacologically inactive morphine-3-glucuronide (M3G), and the pharmacologically active morphine-6-glucuronide. The latter is normally not found in significant quantities in rodents (Morland et al., 1994; Milne et al., 1996; Grung et al., 1998).

Studies in the 1980s suggested that heroin is a prodrug acting through its metabolites (Inturrisi et al., 1983). Previous work in our laboratory has shown that 6-MAM is the predominating metabolite in brain and blood after subcutaneous and intravenous heroin administration in mice and rats, respectively, indicating that 6-MAM is the main contributor to acute heroin effects (Andersen et al., 2009; Gottås et al., 2013). In addition, the psychomotor-stimulating effect and dopamine peak levels coincided with the rising concentration of 6-MAM in brain tissue, at a time when heroin was no longer detectable (Andersen et al., 2009; Gottås et al., 2013, 2014). Furthermore, pharmacokinetic studies have indicated that heroin is mainly metabolized outside the brain (Boix et al., 2013).

On the assumption that 6-MAM is the principal contributor to acute heroin effects and is delivered from the blood, we showed previously that a human mAb against 6-MAM (anti-6-MAM mAb) markedly reduced acute locomotor activity and the brain 6-MAM concentration after heroin injection in mice (Bogen et al., 2014). To develop efficient mAbs for immunotherapy against heroin, we need a better understanding of the individual contributions of heroin and its metabolites to the acute effects upon administration. The aim of the present study was to characterize the pharmacological potential of anti-6-MAM mAb against acute heroin effects and to examine the individual contributions from heroin and 6-MAM to the heroin response.

Materials and Methods

Animals. Male C57BL/6J mice (7–8 weeks old, 20–25 g; Taconic, Ejby, Denmark) were housed four to eight per cage in the animal facility at the Norwegian Institute of Public Health (22 ± 1°C, 50 ± 10% humidity, light period 7 AM–7 PM). The animals were acclimatized for at least 5 days prior to the experiments. Commercial mouse pellets and water were available ad libitum. All experimental materials for at least 5 days prior to the experiments. Commercial mouse pellets and water were available ad libitum. All experimental materials and procedures were approved by the Norwegian Animal Research Authority.

Materials. Antibody: Anti-6-MAM mAb (human immunoglobulin G1 [IgG1]) was provided by Affitech Research AS (Oslo, Norway) and has been described in more detail by Bogen et al. (2014) and Moghaddam et al. (2003). The mAb was dialyzed against phosphate buffer, diluted in 0.9% NaCl, and stored at −80°C. Drugs: Heroin-HCl (mol. wt. 421.91) and 6-MAM-HCl (mol. wt. 182) were purchased from Lipomed AG (Arlesheim, Switzerland) and stored at −80°C. Blood samples for antibody IgG1 quantification by enzyme-linked immunosorbent assay (ELISA) were immediately frozen in liquid nitrogen and stored at −80°C. Blood samples (100 µl) for opioid analysis were collected in 5-ml tubes containing 100 µl ice-cold ammonium formate buffer (5 mM, pH 3.1) with sodium fluoride (4 mg/ml) and immediately frozen in liquid nitrogen. The samples were stored at −80°C until opioid analysis by liquid chromatography–tandem mass spectrometry (LC-MS/MS) within 24 hours.

Sample Preparation of Blood for Opioid and Antibody Analysis. Blood sampling by heart puncture was performed 5, 10, or 25 minutes after heroin or 6-MAM injection (1.25 or 2.5 µmol/kg, s.c.) by using a syringe needle prefilled with 50 µl heparin (100 IU/ml). Blood samples for antibody IgG1 quantification by enzyme-linked immunosorbent assay (ELISA) were immediately frozen in liquid nitrogen and stored at −80°C. Blood samples (100 µl) for opioid analysis were collected in 5-ml tubes containing 100 µl ice-cold ammonium formate buffer (5 mM, pH 3.1) with sodium fluoride (4 mg/ml) and immediately frozen in liquid nitrogen. The samples were stored at −80°C until opioid analysis by liquid chromatography–tandem mass spectrometry (LC-MS/MS) within 24 hours.

Sample Preparation of Brain Tissue for Opioid and Antibody Analysis. After blood sampling and cervical dislocation, the cerebrum was quickly removed, washed in ice-cold 0.9% NaCl, and blotted on filter paper. One hemisphere was homogenized (4 ml/g tissue) in ice-cold ammonium formate buffer (5 mM, pH 3.1) containing sodium fluoride (4 mg/ml) and immediately frozen in liquid nitrogen. The samples were stored at −80°C until opioid analysis by LC-MS/MS within 24 hours, or until antibody IgG1 quantification by ELISA.

Opioid Analysis. Heroin and heroin metabolites were quantified by a LC-MS/MS method previously described by Karinen et al. (2009). Briefly, internal standard (50 µl, final conc. 0.5 µM) and ice-cold acetonitrile/methanol (500 µl, 85:15) were added to brain homogenate or blood samples (200 µl), immediately shaken, and frozen for at least 10 minutes at −20°C. After centrifugation (3900 g 4°C, 10 minutes), the supernatant was transferred to glass tubes and evaporated to dryness at 40°C under a gentle stream of nitrogen. The dry residue was reconstituted with ice-cold mobile phase (100 µl, 3% acetonitrile/97% 5 mM ammonium formate buffer, pH 3.1) and centrifuged (3900 g 4°C, 10 minutes). The resulting supernatants were transferred to autoinjector vials. Separation of samples was performed at 50°C on an XTerra MS C18 column (Waters Corp., Milford, MA) using gradient elution with mobile phase consisting of methanol and ammonium formate buffer (5 mM, pH 3.1) and a flow rate of 0.2 ml/min. The limits of detection in brain tissue were 0.005 nmol/g for morphine, 0.003 nmol/g for 6-MAM, and 0.008 nmol/g for heroin. The limits of detection in blood were 0.0133 nmol/g for M3G, 0.0014 nmol/g for morphine, 0.0008 nmol/g for 6-MAM, and 0.0023 nmol/g for heroin. Data acquisition, peak integration, and quantification of samples were performed using MassLynx 4.0 SCN509 software (Waters Corp., Milford, MA).

Antibody IgG1 Quantification. Blood and brain samples were diluted and analyzed for human IgG1 using a Novex ELISA kit (Thermo Fisher Scientific Inc., Waltham, MA). Two duplicates of each standard and control were used. Absorbance was measured (450 nm) within 1 hour of adding the stop solution using an ELX808 Absorbance Microplate Reader (BioTek Instruments Inc., Winooski, VT).

Data and Statistical Analysis. Data are presented as mean ± S.E.M unless stated otherwise. To ease the comparison between blood and brain values, all opioid concentrations are expressed as nmol/g, which approximately equals the nmol/ml concentration in blood (blood density 1.06 mg/ml). Each dataset was assessed for normal distribution using a one-sample Kolmogorov-Smirnov or a Shapiro-Wilk test. The data were to a large extent not normally distributed, therefore a nonparametric statistical test was performed (Mann-Whitney U test). P values less than 0.05 were considered statistically significant. All statistical tests were performed using SPSS, version 20 (SPSS Inc., Chicago, IL).
Results

Following mAb or saline pretreatment (controls), all mice received a single heroin or 6-MAM injection. Unless otherwise stated, the locomotor activity and opioid concentrations were measured for 20 and at 25 minutes after opioid injection, respectively.

Initially, the optimal time interval between mAb pretreatment and heroin administration in mice was examined. mAb pretreatment 1–2 hours prior to heroin injection significantly reduced locomotor activity by 44–60% (P < 0.01; Fig. 1A), and brain 6-MAM levels by 24–43% (P < 0.05; Fig. 1B), compared with controls. The maximum efficacy of the mAb on the heroin effect was observed 4–72 hours after mAb pretreatment, with a 52–66% reduction in heroin-induced locomotor activity (P < 0.01; Fig. 1A), and a 55–59% reduction in brain 6-MAM levels, compared with controls (P < 0.001; Fig. 1B). At 96 hours following mAb pretreatment, the locomotor activity and 6-MAM levels in brain tissue after heroin injection were still reduced by 36 and 51%, respectively (P < 0.01; Fig. 1A and B). The blood concentrations of human IgG1 measured 4–72 hours after mAb pretreatment (10 mg/kg) ranged from 105 ± 2 to 125 ± 8 μg/ml, whereas 96 hours after mAb treatment the IgG1 concentration was 87 ± 7 μg/ml (Fig. 1C). The highest human IgG1 level in mouse blood was measured 48 hours after mAb injection and was significantly higher (30%) compared with 96 hours following mAb pretreatment (P < 0.05; Fig. 1C). All subsequent experiments in the present study were performed by pretreating mice with anti-6-MAM mAb 48 hours prior to opioid administration.

Next, we compared the effect of anti-6-MAM mAb pretreatment (10 mg/kg) on the acute behavioral response and pharmacokinetics after heroin and 6-MAM administration (2.5 μmol/kg). Mice pretreated with mAb prior to heroin injection had a 60% reduced locomotor activity measured 20 minutes after injection, and a 37% reduction in total run distance compared with controls (P < 0.05; Fig. 2, A and C). mAb pretreatment prior to 6-MAM injection reduced locomotor activity measured 20 minutes after injection by 69% and total run distance by 40%, compared with controls (P < 0.05; Fig. 2, B and D). E_max was reached earlier for mAb-pretreated mice (8 minutes) versus controls (12–14 minutes), after both heroin and 6-MAM administration. In control animals, heroin injection caused an E_max of 909 ± 69 cm2/minute (Fig. 2A), whereas an equimolar dose of 6-MAM resulted in an E_max of 676 ± 63 cm2/minute (Fig. 2B). The total run distance (cm/20 minutes) was 35% higher after heroin injection compared with an equimolar dose of 6-MAM (P < 0.01; Fig. 2, C and D).

Heroin was detectable in blood and brain tissue only at 5 minutes following injection (2.5 μmol/kg), and the presence of mAb (10 mg/kg) did not significantly affect heroin levels (inserts Figs. 3A and 4A). Five minutes after heroin injection, brain levels of 6-MAM were not significantly reduced in mAb-pretreated mice compared with controls (Fig. 3A). Ten and 25 minutes after heroin injection, the brain 6-MAM levels were reduced by 32% (P < 0.05) and 56% (P < 0.001), respectively, in mAb-pretreated mice compared with controls (Fig. 3A). Five minutes after heroin injection, there was no difference in brain morphine levels between mAb-pretreated mice and controls (Fig. 3C). However, the morphine concentration in brain tissue was reduced by 32 and 33%, respectively, 10 and 25 minutes after heroin injection in mAb-pretreated mice (P < 0.05; Fig. 3C).

mAb pretreatment prior to 6-MAM injection reduced the brain 6-MAM level by 57% compared with controls already at 5 minutes after injection (P < 0.01), and the concentration was reduced by 56% (P < 0.01) 10 minutes, and 68% (P < 0.001) 25 minutes after injection (Fig. 3B). Only trace amounts of morphine were found in brain tissue 5 minutes after 6-MAM injection, for both controls and mAb-pretreated mice. At 10 and 25 minutes after 6-MAM injection, the brain morphine concentration was reduced by 93 and 78%, respectively, in mAb-pretreated mice compared with controls (P < 0.01; Fig. 3D).

In general, an injection of heroin (2.5 μmol/kg) caused significantly higher concentrations of 6-MAM and morphine
in brain tissue compared with an equimolar dose of 6-MAM, both in the presence and the absence of mAb (Fig. 3, A–D). When comparing saline-pretreated controls, brain levels of 6-MAM were 0.81 ± 0.14 and 0.45 ± 0.06 nmol/g measured 5 minutes after an injection of heroin and 6-MAM, respectively. Ten minutes after heroin or 6-MAM injection, 6-MAM levels in brain tissue from control animals were 0.97 ± 0.06 nmol/g and 0.71 ± 0.07 nmol/g, respectively (*P < 0.05; Fig. 3, A and B).

Anti-6-MAM mAb (10 mg/kg) had no significant effect on 6-MAM levels in blood after heroin or 6-MAM injection (2.5 μmol/kg), with the exception of a 29% reduction measured 25 minutes after 6-MAM injection (*P < 0.05; Fig. 4B). There were no differences in blood 6-MAM levels in control mice after injection of heroin compared with 6-MAM (Fig. 4, A and B). For mAb-pretreated mice, 41 and 48% decreases in blood morphine concentration were found 25 minutes after heroin and 6-MAM injection, respectively (*P < 0.05; Fig. 4, C and D). The blood M3G concentration of mAb-pretreated mice was doubled compared with controls (P < 0.05; Fig. 4, E and F).

The acute behavioral response and pharmacokinetics after heroin and 6-MAM administration were further examined by increasing the administered mAb dose. In these experiments, mice were pretreated with 10–100 mg/kg mAb, followed by single heroin or 6-MAM injections (2.5 μmol/kg). Pretreatment with 10, 50, or 100 mg/kg mAb reduced locomotor activity measured 20 minutes after heroin injection by 60, 82, and 83%, respectively, compared with saline-pretreated controls (P < 0.001; Fig. 5A). In comparison, locomotor activity after 6-MAM injection was reduced by 69, 93, and 100% after 20 minutes (P < 0.001; Fig. 5B). Mice pretreated with 10, 50 or 100 mg/kg mAb, had a 56, 79, and 77%, reduction in brain 6-MAM concentration after heroin administration (*P < 0.001; Fig. 5C). The 6-MAM concentration measured in brain 25 minutes after 6-MAM injection was reduced by 68, 88, and 90% in mice pretreated with 10, 50, and 100 mg/kg mAb, respectively (P < 0.001; Fig. 5D). mAb-pretreated mice (10–100 mg/kg) had a 30–51% and 47–81% reduction in brain morphine levels after heroin and 6-MAM injection, respectively (*P < 0.01; Fig. 5, E and F). The human IgG1 levels measured in mouse blood 48 hours following pretreatment with 10, 50, or 100 mg/kg mAb were 125 ± 8, 600 ± 60, and 1150 ± 86 μg/ml, respectively (Fig. 5G). No human IgG1 was found in blood from saline-pretreated animals, or in brain tissue from mice pretreated with mAb (not shown).

To enhance the blockade of immediate heroin/6-MAM effects, we examined various ratios of opioid versus mAb binding sites administered. For this purpose mice were given mAb doses of 10–100 mg/kg and opioid doses of 1.25 and 2.5 μmol/kg. The resulting 6-MAM levels measured in brain and blood 5 and 25 minutes after opioid injection are presented as percentage of control in Table 1. Heroin/mAb ratios of both 19 and 1 resulted in nonsignificantly reduced brain 6-MAM concentrations (73% and 63% of control, respectively) 5 minutes after heroin injection. By changing the heroin/mAb ratio from 19 to 2, the brain 6-MAM levels 25 minutes after heroin injection decreased from 44% (*P < 0.01) to 23% (*P < 0.001) of control levels. In comparison, by changing the 6-MAM/mAb ratio from 19 to 1 or 2, brain 6-MAM levels decreased from 43% (*P < 0.01) to 26% (*P < 0.05) 5 minutes after 6-MAM injection, and from 32% (*P < 0.001) to 10% (*P < 0.001) 25 minutes after injection. An opioid/mAb ratio of 1 resulted in 3.3-fold (330%; *P < 0.01) and 3.1-fold (307%; *P < 0.05) increases in blood 6-MAM levels measured 5 minutes after injection of heroin.
and 6-MAM, respectively. Employing heroin/mAb ratios of 2 to 19 had no significant effect on blood 6-MAM levels measured 5 and 25 minutes after injection. However, 6-MAM/mAb ratios of 19 and 4 significantly changed 6-MAM levels in blood to 71% ($P < 0.05$) and 42% ($P < 0.01$) of control levels, respectively.

**Discussion**

In the present study, we have characterized the pharmacological potential of a monoclonal antibody against 6-MAM to block the acute heroin effect. The individual contributions from heroin and 6-MAM were examined by pretreating mice with anti-6-MAM mAb prior to either heroin or 6-MAM injection. The main findings of this study were: 1) pretreatment with anti-6-MAM mAb caused a substantial reduction of heroin- and 6-MAM-induced effects; 2) a more prominent effect of mAb pretreatment was observed upon 6-MAM injection compared with heroin injection; and 3) although 6-MAM is the main contributor to the acute heroin response, heroin itself appears to enhance the immediate delivery of 6-MAM to the brain.

In rodents, striatal dopamine release after opioid injection produces psychomotor stimulation, which is commonly used as a behavioral measure for opioid reward (Beninger, 1983; Wise and Bozarth, 1987; Mørland et al., 1994; Fields and Margolis, 2015). Here, we report a considerable reduction in locomotor activity and 6-MAM levels in brain tissue after both heroin and 6-MAM administration in mAb-pretreated mice. These findings agree with previous reports that 6-MAM is the main contributor to the acute behavioral effects of heroin (Way et al., 1960; Inturrisi et al., 1983; Andersen et al., 2009; Gottâs et al., 2013), and that 6-MAM sequestration is crucial for the efficacy of immunotherapy against heroin effects (Stowe et al., 2011; Bogen et al., 2014).

The observed effect of anti-6-MAM mAb was most prominent 25 minutes after opioid injection, with dose-dependent reductions of brain 6-MAM levels from 44 to 23% and 32 to 10% of control levels after a single injection of heroin and 6-MAM, respectively (Table 1). The reductions in brain 6-MAM levels closely corresponded to the reduced locomotor activity response observed for both opioids. To study the individual contributions to the immediate heroin effect, we also examined the early pharmacokinetic profiles of injected heroin and 6-MAM. Within 5 minutes after heroin injection, an almost complete conversion to 6-MAM had occurred, as could be read from the very low heroin concentrations in blood and brain. Using a heroin/mAb binding sites ratio of 1, we observed a tendency toward reduced brain 6-MAM concentration (37%, not significant) 5 minutes after injection. In contrast, a 74% ($P < 0.05$) reduction in brain 6-MAM concentration was found immediately following 6-MAM injection. In previous studies using active immunization against heroin effects in rats, 44% and 69% reductions in brain 6-MAM levels were found 4 minutes after a single heroin or 6-MAM injection, respectively (Pravetoni et al., 2012; Raleigh et al., 2013). Another study by Raleigh et al. (2014) reported 26% (not significant) and 48% reductions in brain 6-MAM levels 4 minutes after a series of heroin infusions, using final drug/mAb binding sites ratios of 4 and 2. Thus, our current findings employing a mAb toward 6-MAM closely correlate with those of other studies using active vaccination.
The anti-6-MAM mAb used in this study displayed high specificity for 6-MAM, lower specificity for heroin, and no specificity for morphine (Bogen et al., 2014). One may question whether the immediate heroin effect could have been inhibited more successfully by using a mAb with high specificity for heroin. The active vaccine employed in the studies mentioned above (Pravetoni et al., 2012; Raleigh et al., 2013, 2014) generated antibodies with equal specificity for both heroin and 6-MAM. Although their active vaccine seemed slightly more effective in reducing the immediate brain 6-MAM concentration, as with our mAb their vaccine did not affect the brain heroin levels in immunized animals. These results imply that the antibodies with high heroin specificity were unable to block the very rapid transport of heroin across the BBB.

One advantage with passive immunization is that the mAb dose can easily be adjusted to counteract high heroin doses. Therefore, we examined the ratio of mAb versus drug dose needed to significantly reduce the heroin and 6-MAM-induced effect. Decreasing the drug/mAb ratio improved mAb efficacy upon both heroin and 6-MAM injection. However, whereas 6-MAM/mAb ratios of 2 and 4 inhibited the 6-MAM-induced locomotor activity almost completely, and reduced brain 6-MAM levels by 71–90% mAb pretreatment was less efficient against heroin. Although heroin metabolizes extremely fast in mouse blood (Boix et al., 2013), our data indicate that a fraction of the injected heroin enters the brain so rapidly that it escapes both metabolism and retention by antibodies in the blood.

The negligible heroin levels in brain shortly after injection found in the current and other studies are presumably not responsible for the behavioral effect observed at this time (Andersen et al., 2009; Gottás et al., 2013). Nevertheless, both...
in the presence and absence of mAb we report a more profound psychomotor effect and higher 6-MAM levels in brain after injection of heroin compared with 6-MAM, as previously indicated after both subcutaneous and intravenous administration (Andersen et al., 2009; Gottás et al., 2014). Heroin itself is highly lipophilic and may rapidly cross the BBB for subsequent deacetylation into 6-MAM (Oldendorf et al., 1972; Umans and Inturrisi, 1981). Although in vitro heroin metabolism in brain tissue homogenate has been estimated as rather slow compared with that in blood (Boix et al., 2013), both neurons and non-neuronal cells display esterase activity capable of transforming heroin into 6-MAM (Tower and

Fig. 5. (A, B) Locomotor activity, (C–F) brain opioid concentrations, and (G) blood IgG1 concentrations after a single injection of either heroin (left panel) or 6-MAM (right panel) (2.5 μmol/kg, s.c.) in mice pretreated with anti-6-MAM mAb (10, 50, or 100 mg/kg, i.p.) or saline (7 ml/kg, i.p) 48 hours prior to opioid injection. Locomotor activity and brain opioid concentrations were measured the first 20 minutes and at 25 minutes after opioid injection, respectively, n = 3–13. Values are expressed as mean ± S.E.M.; Mann-Whitney U test; **P < 0.01, ***P < 0.001. In Fig. 5, A and B), statistical symbols are omitted for clarity.
Young, 1973; Tago et al., 1992; Reid et al., 2013). Previous studies have demonstrated human brain esterase activity at the endothelial component of the BBB (Mori et al., 1999; Darreh-Shori et al., 2013), implying that heroin might be hydrolyzed into 6-MAM upon brain entrance. This could explain the rapid increase in 6-MAM brain levels shortly after heroin injection, producing the more potent psychomotor effect compared with injected 6-MAM.

Studies employing active vaccination against heroin have reported substantially increased blood 6-MAM levels (5–1000-fold) in immunized rats (Pravetoni et al., 2012; Raleigh et al., 2013; Schlosburg et al., 2013). In the present study, a ratio of 1 between mAb binding sites and opioid elicited a 3-fold increase in blood 6-MAM levels, which is in accordance with previous studies (Schlosburg et al., 2013; Raleigh et al., 2014) and demonstrates drug sequestration in the blood. However, a drug/mAb ratio of 19 did not increase blood 6-MAM concentrations; rather, it reduced morphine and elevated M3G levels, indicating an escalated morphine metabolism in the presence of mAb.

The human IgG1 concentrations measured in blood after mAb pretreatment were consistent with theoretical concentrations, calculated using a distribution volume of 132 ml/kg in rats (Bazin-Redureau et al., 1997). As expected, human IgG was not detected in brain tissue of mAb-pretreated mice, confirming the principles for immunotherapy toward drug abuse (Brightman et al., 1970; Seitz et al., 1985; Tabrizi et al., 2010; Pardridge, 2012). We explored different time intervals between mAb and heroin administration in mice and found 4–48 hours to be optimal. The IgG levels in brain remained stable for at least 72 hours, but a tendency toward lower mAb efficacy was observed after 96 hours, in accordance with its previously reported biologic half-life of 8–9 days (Bogen et al., 2014). The half-life of IgGs in humans is several weeks and may be engineered to be even longer, making feasible the design of mAb therapy for long-lasting effects against heroin in humans (Peterson and Owens, 2009).

An advantage of immunotherapy is that immunoglobulins are highly specific, they do not cross the BBB, and therefore do not interfere with the effects of endogenous opioids or opioids used in substitution therapy. A rapid onset of action provides potential for mAbs in acute heroin overdose reversal, as a supplement to existing short-lasting treatment such as naloxone (Kosten and Owens, 2005; Janda and Treweek, 2011; Brimijoin et al., 2013). Furthermore, specific antibodies may be tailored for the respective patient, including precise dosage and predictable duration of action (Peterson and Owens, 2009). Passive immunization may be particularly useful during pregnancy, and after rehabilitation or prison release owing to the high risk of relapse. An immunotherapeutic approach against heroin also comprises challenges, including high-cost production of large quantities of mAbs, the need of repeated injections to sustain long-lasting antidrug effects, and highly motivated patients (Brimijoin et al., 2013).

The current study shows that immunotherapy against heroin has promising pharmacological potential. We provide new knowledge on the pharmacokinetics underlying the acute heroin effect, which is important for developing efficient immunotherapy against heroin. Sequestration of 6-MAM in the blood prior to brain entrance is crucial, as it is the main contributor to the acute heroin response. Considering that heroin itself appears to enhance the delivery of 6-MAM to the brain, one impending challenge with immunotherapy design against heroin is to block the most immediate heroin effect.

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Authorship Contributions

Participated in research design: Kvello, Andersen, Øiestad, Mørland, Bogen.

Conducted experiments: Kvello, Andersen, Bogen.

Performed data analysis: Kvello, Andersen, Bogen.

Wrote or contributed to the writing of the manuscript: Kvello, Andersen, Øiestad, Mørland, Bogen.

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