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Chronic anti-phencyclidine monoclonal antibody therapy decreases phencyclidine-induced *in utero* fetal mortality in pregnant rats

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Abstract

Illicit drug use during pregnancy is a serious social and public health problem inflicting an array of deleterious effects on both mother and offspring. We investigated the hypothesis that a murine anti-phencyclidine (PCP) monoclonal antibody (mAb6B5; $K_D=1.3$ nM) can safely protect mother and fetus from PCP-induced adverse health effects in pregnant rats. Pregnant Sprague-Dawley rats ($n=4-5$) were intravenously administered bolus injections of PCP (1 mg/kg) on multiple days during pregnancy. They were also chronically treated with anti-PCP mAb6B5 at 45 mg/kg as a PCP antagonist. This dose provided one mAb-PCP binding site for every four PCP molecules. Therapeutic and safety study endpoints included pregnancy outcome (litter size, number of live vs. dead pups), maternal hemodynamic status and locomotor activity. Maternal hemodynamic changes (*i.e.*, blood pressure and heart rate) and locomotor activity were measured in dams from gestation days 6–21 (one day antepartum) using a radiotelemetry-tracking device with a femoral arterial pressure catheter. This mAb6B5 treatment regimen significantly ($p=0.008$) reduced the number of PCP-induced *in utero* fetal deaths (odds ratio=3.2; 95% CI 1.3 to 7.9) and significantly ($p<0.05$) reduced acute PCP-induced maternal locomotor effects in the second trimester. Maternal hemodynamic responses to PCP were not significantly affected by mAb6B5 treatment. In conclusion, these data suggest that anti-PCP mAb treatments administered during pregnancy can safely protect a mother and her fetus(es) from PCP-related morbidity and mortality even when the mAb dose is too low to significantly prevent other PCP-induced maternal pharmacological effects.

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1. Introduction

Preclinical and clinical studies show that antibodies from passive and active immunization have been used to prevent adverse medical effects from small molecules (e.g. <750 Da), including highly addictive drugs of abuse [1–6]. The United Nations and World Health Organization report illicit drug use continues to increase and new, better medications are needed to combat the resulting social, economic, and medical impact [7]. Monoclonal antibody (mAb) medications against these small molecule chemical represent a relatively new class of medication possessing characteristics and mechanisms of action that are in some ways ideal for treating drug abuse [8].

Anti-drug mAbs work by reducing the dose/concentration of target ligands in vulnerable organs like the brain [8–12]. MAbs primarily mediate these therapeutic benefits from the blood stream, without entering the central nervous system (CNS). MAbs also avoid the addiction potential and other complications inherent with small molecule CNS-receptor agonist/antagonist medications (e.g., methadone) [11–13]. In addition, the long terminal elimination half-life ($t_{1/2\lambda z}$) of IgG mAbs (~21 days in humans) could allow maintenance mAb medication dosing once every 2–4 weeks [14, 15], in contrast to small molecule agonist or antagonist treatments, which require dosing 1–3 times per day. This greatly extended dosing interval could potentially improve patient adherence to treatment plans.

Pregnant women and their unborn children are important patient populations that could benefit from effective treatments for drug abuse and addiction [7]. To date, pre-clinical models of mAb medications for drug abuse treatment have employed a diverse set of therapeutic endpoints to evaluate mAb protection, including general health (e.g., weight, survival), brain drug concentrations, behavioral effects (e.g., locomotor activity, self-administration behavior), and cardiovascular measures (e.g., heart rate, blood pressure) [1, 9, 16]. Together, these studies show that drug-specific mAbs can favorably alter drug disposition across the blood-brain barrier and thereby prevent or ameliorate many adverse drug effects.

During pregnancy, the blood-placenta barrier provides another interface at which mAb medications could potentially antagonize the pharmacokinetic properties of drugs of abuse and thereby decrease fetal exposure resulting maternal use of these chemicals. Previous work in acute and chronic nicotine abuse models in pregnant rats shows anti-nicotine antibody medications (both mAbs and polyclonal antibodies) can decrease fetal brain nicotine levels late in pregnancy. While physiologic endpoints and health outcomes were not determined [17–19], these studies demonstrate a promising potential for mAb therapy for drug abuse during pregnancy, provided it is safe for both mother and fetus.

Phencyclidine (PCP) is an illicit drug with serious adverse effects [20]. PCP abuse, which is most common among young adults of childbearing age, is usually characterized by occasional abuse of large doses in an acute, binge manner [21]. PCP readily crosses rat and human placentas, and human fetal PCP concentrations greatly exceed maternal levels [22–24]. This leads to adverse fetal effects, including intrauterine growth retardation and neurobehavioral effects (e.g., irritability, nervousness, and hypertonicity) [25, 26]. In rats, PCP-exposed fetuses suffer increased neuroapoptosis, decreased motor coordination, and hyperactivity [25, 26]. PCP also causes extensive neurologic and cardiovascular effects in humans, including dose-dependent dissociative analgesia and anesthesia with associated ataxia and dysphoria [20, 27, 28]. PCP and its close relative ketamine also directly stimulate the sympathetic nervous system resulting in increased heart rate and blood pressure [20, 27].

We previously demonstrated in male rats that (1) anti-PCP mAb6B5 can provide long-term protection against PCP-induced health and behavioral effects [10], (2) this protection occurs

at very low mAb doses (1/100th mole-equivalents to the PCP body burden at steady-state) [10], and (3) protection can persist in spite of attempts to surmount the beneficial effects of mAb treatment with intravenous (IV) bolus doses of PCP [29]. We also find mAb pharmacokinetics and pharmacodynamics fluctuate in a novel manner during each trimester of rat pregnancy, but an adaptive dosing schedule can maintain mAb-mediated reductions in maternal and fetal brain levels even during chronic PCP-dosing [30].

The current studies utilized a pregnant rat model of acute, binge PCP abuse to test the hypothesis that anti-PCP mAb6B5 can be protective and safe for mother and fetus in a clinically relevant drug abuse model. For these studies, we administered repeated mAb6B5 doses to pregnant rats along with high-doses of PCP, and measured pregnancy outcomes and maternal physiologic effects (e.g., hemodynamic and locomotor response) as pharmacological and safety endpoints. While chronic mAb6B5 treatment appeared safe for mother and fetus, it only modestly reduced maternal locomotor effects in the second trimester and did not significantly alter maternal hemodynamic effects of PCP. Importantly, mAb6B5 treatment significantly protected against *in utero* fetal death from acute maternal PCP exposure.

2. Methods

2.1 Materials

PCP-HCl [1-(1-[phenylcyclohexyl] piperidine hydrochloride] and [³H]-PCP [1-(1-[phenyl-³H](*n*)cyclo-hexyl)piperidine] were obtained from the National Institute on Drug Abuse (Bethesda, MD), and [1-(1-(phenyl-*d*₅)cyclohexyl) piperidine] (PCP-*d*₅), from Sigma/Isotec (Miamisburg, OH). Normal rat serum (NRS) was from Pel-Freez Biologicals (Rogers, AR). All other reagents were from Thermo Fisher Scientific Co. (Fairlawn, NJ), unless otherwise specified. Water used for all buffers was purified with a Milli-Q Synthesis A10 System (Millipore; Bedford, MA).

2.2 Monoclonal antibodies

The anti-PCP mAb6B5 used in these studies was produced as previously described [6, 10, 31], with minor modifications. Murine hybridoma cell line mAb6B5 was used to produce gram quantities of anti-PCP IgG₁ (κ-light chain) mAb6B5 in a Wave Bioreactor (GE Healthcare, Piscataway, NJ). MAb6B5 was purified by protein G affinity chromatography from bioreactor media, and then concentrated to ~30 mg/ml in mAb administration vehicle (15 mM sodium phosphate, 0.15 M sodium chloride, pH 6.5). MAb stocks were stored at -80°C until use, when the appropriate volume was thawed, centrifuged first for 1 h at 100,000 g and 4°C, then for 20 min at 3,000 g and 4°C. Final dosing preparations were made by diluting mAb in mAb administration vehicle using aseptic technique.

2.3 Animals

All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the National Institutes of Health, and were performed with the prior approval of the Animal Care and Use Committee of the University of Arkansas for Medical Sciences. Female Sprague-Dawley (SD) rats (225–250 g; age=65–85 days) were purchased from Charles River Laboratories (CRL, Raleigh, NC). All rats were impregnated, operated upon, and shipped on the same schedule, with impregnation occurring on GD0, surgery on GD1, shipping on GD3 from CRL, and arrival at the University of Arkansas for Medical Sciences on GD4.

Impregnation, jugular venous catheterization (JVC; using Silastic medical-grade tubing, 0.020" inner diameter and 0.037" outer diameter; Dow Corning, Midland, MI), and

radiotelemetry implantation (using PA-C40 transmitters, Data Sciences International, St. Paul, MN) procedures were performed by CRL on gestation day 1 (GD1) before shipping. Jugular catheterization and radiotelemetry implantation were performed simultaneously. The radiotelemetry implants consisted of an arterial catheter inserted into the femoral artery, with the catheter tip lying in the abdominal aorta caudal to the renal artery bifurcation. The transmitter body was placed subcutaneously on the left flank just rostral to the arterial catheter entry point by CRL surgeons. At the University of Arkansas for Medical Sciences, rats were housed individually in the same room used for studies, which provided a light- and temperature-controlled environment (12 h light/dark cycles). All rats were fed/watered *ad libidum*.

Acclimation of the rats to handling began on the day of arrival by daily weighing and JVC-handling. Flushing/filling catheters with normal saline post-mAb dosing maintained catheter patency, and, if resistance was detected at dosing, catheters were filled with 0.05 ml of glycerol/heparin solution (500 USP units heparin/ml). No blood samples were collected during this study. Rats were observed daily for changes in weight, general appearance and the presence of chromodacryorrhea (red lacrimal secretions). Fetal absorptions were determined by standard methods (see [32, 33] for examples) when the pups were delivered by cesarean section.

2.4 Study design

Pregnant rats (n=12) were impregnated and implanted with subcutaneous hemodynamic monitoring devices prior to the start of the study. The pregnant rats (n=6 per group) were divided into a control group treated with PCP and vehicle (i.e., mAb6B5 formulation buffer), and a mAb treatment group treated with PCP and mAb6B5.

Figure 1 shows the dosing of PCP and mAb6B5 over the entire course of the experiment. PCP for IV bolus dosing was prepared using normal saline at a dose of 1 mg/kg PCP-HCl. This dose was the ~LD₅ (5% lethal) in male rat studies [29], and elicited significant hemodynamic and locomotor responses in preliminary hemodynamic experiments (n=2) in pregnant female rats. This PCP dose also represents a 4-fold molar excess of PCP relative to the mAb-antigen binding site dose provided by the 45 mg/kg mAb6B5 dosing regimen described below. Previous studies of acute PCP administration to male rats show that locomotor responses to PCP after an initial priming dose are consistent for the second and all subsequent PCP doses [34]. Therefore, the present studies incorporated a priming dose of 1 mg/kg PCP-HCl on GD6, followed by additional study doses on GD7, GD9, GD12, GD18, and GD21 (Fig. 1). PCP-induced changes in the cardiovascular parameters (blood pressure and heart rate), as well as locomotor activity, maternal health, and pregnancy outcomes served as safety and therapeutic endpoints.

Anti-PCP MAb6B5 was administered on a repeated dosing regimen: one dose every mAb $t_{1/2\lambda_z}$, meaning once every 3 days during the 2nd trimester and once every day during the 3rd trimester (Fig. 1) [30]. The initial mAb dose was a loading dose (D_L) calculated using the equation $D_L = R \cdot D_m$, where D_m is the maintenance dose and R is the accumulation index. The accumulation index (R) was calculated according to the equation, $R = 1/(1 - e^{-\lambda_z \tau})$, where e = root of the natural log, λ_z = terminal elimination rate constant, τ = dosing interval (in days). The D_L and D_m were 90 and 45 mg/kg of mAb6B5, respectively.

MAb6B5 doses were aseptically prepared in 1 ml volumes and administered over 30–45 seconds. Controls received 1 ml vehicle without mAb. Rats received either mAb6B5 or vehicle on GD8, GD11, GD14, and once every day from GD16–GD21. On PCP-dosing days, each mAb6B5 dose was administered approximately one-third of a mAb6B5 half-life (24 h in the 2nd trimester, and 8 h during the 3rd trimester) before the PCP dose. This dosing

schedule ensured that, at the time of PCP administration, each rat had ~70 mg/kg of mAb6B5 present, according to the equation $A = D \times e^{(-\lambda z \times t)}$, where A = amount of the dose remaining, D = the dose administered, e = the root of the natural log, and t = the time from the last dose (in days). Thus, A in these experiments (~70 mg/kg) represents a molar equivalent of PCP-binding sites to PCP of 1:4.

2.5 Hemodynamic and locomotor measurements

Timed pregnant rats underwent surgery on GD1 (performed by the animal vendor, Charles River Laboratories, Raleigh, NC) to implant radiotelemetry transmitters and an arterial catheter capable of measuring blood pressure, heart rate and locomotor activity. Hemodynamic measurements were collected using PhysioTel® telemetry RPC-1 receivers, with DATAquest ART version 4.01 (Data Sciences International) software for data recording and analysis. The animals were shipped from the vendor on GD3 and received on GD4. Maternal weight was measured every day ≥ 2 h prior to baseline measurements. Animal husbandry duties (i.e., cage cleaning, feeding, watering) were completed at least 24 h before hemodynamic and locomotor baseline measurements began. Procedures performed on each PCP-dose day (GD7, GD9, GD12, GD18 and GD21) were scheduled relative to PCP dosing (defined as time 0). Accordingly, baseline measurements were begun at -4 h, followed by saline response (0.1 ml/kg; at -1.5 h), and PCP response (1 mg/kg PCP-HCl; from 0-3.5 h). While the pressure catheter failed in one animal in each group, but the tracking device remained functional in these animals, resulting in $n=4$ /group and $n=5$ /group for HD and activity data, respectively.

2.6 Hemodynamic and locomotor activity data analysis

Hemodynamic data was processed using the DATAquest ART software v4.01 stripping tools (i.e., replacing 0 values with an extrapolated value interpolated from the preceding and following values) and 1 min moving average. Raw measurements of PCP-induced blood pressure (0-70 min) and heart rate responses (10-70 min) from each rat on each PCP dose day were normalized to saline values from each rat by dividing each measurement by the average representative saline response measurements (from -21 to -30 min). Hemodynamic (HD) measures from baseline and saline periods were initially analyzed for day-to-day variance for individual animal and group by visual inspection (within individuals between days). The area under the diastolic BP, systolic BP, pulse pressure, and heart rate-time curves (AUC_D , AUC_S , AUC_{PP} , and AUC_{HR} , respectively) were calculated using Origin™ graphing software (OriginLab Corp., Northampton, MA), which also yielded parameter peak width and time-to-maximum peak height.

To account for the effects of the handling involved in the dosing procedure, each animal in both the vehicle (control) and mAb6B5-treated group received a saline dose at the beginning of each study day. This allowed us to directly assess the effects of PCP on these HD parameters in each group, we normalized the post-PCP measurements to the baseline (i.e., no drug or saline) and saline HD response to isolate PCP-induced changes. Baseline and saline-response measurements on each PCP-dosing day occurred between times -150-120 and -40-10 min, respectively. The effects of the act of dosing *per se* were then subtracted out by normalizing to the saline response as follows: [(PCP-induced response after vehicle or mAb6B5 treatment - response to saline dosing)/response to saline dosing] $\times$ time $\times$ 100% = % Δ *min. Units for BP and heart rate measurements are % Δ mmHg*min and % Δ beats per minute*min, respectively.

Rats were observed after PCP-administration and gross changes in locomotor activity recorded. Locomotor data were processed using the DATAquest ART software v4.01 stripping and 2 min moving average tools. Locomotor activity recorded by the DATAquest

ART software was derived from the signal strength of the telemetry transmission as measured by the receiver antennae, and is a relative measure of the speed and amount of transmitter movement. As such, the activity data collected in this manner could not be directly related to an absolute measure, such as distance traveled (cm). Instead, a measurement (i.e., count) was taken every 10 sec, summed over 1 min periods and reported as counts/min. According to the manufacturer, these activity measurements depend on four factors, including transmitter model (identical for all animals), speed of transmitter movement (animal-dependent), outside influence (e.g., nearby transmitter; controlled for and prevented by transmitter arrangement) and slight receiver-to-receiver variation. Baseline and saline locomotor responses were initially analyzed for variance by visual inspection. PCP-induced responses were analyzed for 80 min periods beginning 8–9 min following PCP dosing, in accordance with previously published locomotor studies [31, 34]. The area under the locomotor-time curve (AUC_L) was calculated using Origin™ graphing software. The change in PCP response from GD7 baseline activity was calculated as for HD parameters.

2.7 Statistical analysis

The categorical data of chromodacryorrhea presence and pregnancy outcome (fetal survival) were analyzed using Fisher's exact test. The HD and locomotor activity parameters were analyzed using two-way, repeated measures ANOVA with Bonferroni post-hoc test. Statistical analysis was performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA). Following study completion, a power analysis was performed with G*power 3.1 software [35] using variance data from the current studies to predict appropriate sample sizes for future studies.

3. Results

3.1 General health

Maternal weight was measured daily throughout pregnancy as a measure of overall animal health. As a percent of GD6 weight, maternal weight gain in pregnant rats from both treatment groups showed a statistically significant weight increase during pregnancy within treatment groups, but weight gains between groups were not different (Fig. 2 upper graph). PCP-induced chromodacryorrhea was negligible in both groups and not significantly different between groups (data not shown, $p=0.37$). Average pup weight (Fig. 2 lower graph) was not statistically different between treatment groups.

3.2 Pregnancy outcome

The overall incidence of *in utero* fetal loss in the vehicle and mAb6B5 treated groups combined was 31 of 146 fetuses, or ~21%. However, mAb6B5/PCP-treated pregnant rats showed significantly ($p=0.008$) fewer *in utero* fetal deaths (8 deaths) than vehicle/PCP-treated controls (23 deaths; Fig. 2). The odds ratio of an *in utero* fetal loss while on PCP without mAb6B5 protection was 3.2 (95% CI 1.3–7.9). Two animals, one each in the vehicle and mAb treatment groups, developed limps in the rear limb where telemetry devices were implanted and were excluded from analysis. Therefore, pregnancy outcome data from $n=5$ per group were analyzed.

3.3 Hemodynamic and locomotor effects of PCP

Two telemetry devices in each group ceased operating during the study. Therefore, hemodynamic and locomotor measurement were completed and analyzed for eight total animals, $n=4$ per group. Each PCP dose (Fig. 1) elicited a significant HD response relative to saline in all parameters measured in both vehicle- and mAb6B5-treated animals. As expected, baseline PCP responses (i.e., without mAb or vehicle on GD7) were not

statistically significant between groups by two-way ANOVA (Fig. 4). The variance in the HD data with only $n=4$ prevented identification of statistically significant differences between mean AUC% changes between days and treatment groups (Fig. 4; Table 1). Locomotor activity effects following PCP dosing were characterized by decreased activity (inability to ambulate), then stereotypy (head-wagging), attempts to ambulate (with initial failure followed by gradual improvement until achieving coordinated ambulation), and, finally, hyperactivity. In particular, some animals were unable to move about their cage immediately following PCP dosing late in pregnancy. These rats fell into a decubitus position upon PCP administration and were temporarily unable to stand or walk, resulting in an initial locomotor activity measurement of zero.

3.4 mAb6B5 protection

Many HD and locomotor parameters appeared similar between treatment groups (Figs 4, Table 1). Importantly, however, the mean time-to-peak locomotor activity was shorter in mAb6B5-treated animals and was statistically significantly different on GD12 ($p<0.05$; Fig. 5 upper panel). Other measures such as the area under the locomotor activity-time curve (AUC_{Activity}; Fig. 5, lower panel), peak height (not shown) and duration of action (not shown), were not significantly different between groups.

3.5 Safety

mAb6B5 treatment did not increase maternal or fetal morbidity or mortality and did not appear to worsen any physiologic or locomotor effects of PCP. However, the nature of our study design did carry some health risk independent of the mAb and PCP administration. One mAb6B5-treated animal developed a limp in the left hind limb (the entry point for the hemodynamic catheter) without signs of infection at necropsy (this animal was excluded from analysis). A vehicle treated animal developed gross hematuria, very weak limbs, lethargy, and weight loss and was also excluded. At necropsy, both animals were negative for gross pathology. Four animals (two from each group) were found to have a localized, subcutaneous, encapsulated abscess (~3.5–4 mm) at the surgical site, without signs of other pathology.

Discussion

The data from these studies demonstrate that anti-PCP mAb6B5 treatment significantly decreased *in utero* fetal death in a clinically relevant animal model of high dose PCP abuse (see Fig. 1 and 3). Specifically, the incidence of *in utero* fetal death in the PCP vehicle-treated rats was high at 29.9% of the fetuses in this group compared to fetal deaths of only 11.6% in the PCP mAb6B5-treated rats. These results provide initial proof of principle supporting the hypothesis that anti-PCP mAb6B5 can be protective for mother and fetus during acute, binge PCP exposure in pregnancy.

One important aspect of mAb-based therapy for drug abuse addressed in this study is the feasibility of this medical strategy. In these studies, an mAb6B5 dose that was four times lower than each dose of PCP was able to reduce the statistical odds of an adverse pregnancy outcome in the presence of PCP (Fig. 3; odds ratio = 3.2, 95%CI 1.3 to 7.9; $p=0.008$). This suggests that higher mAb6B5 doses (i.e., closer to a molar-equivalent of the PCP dose) are not required to achieve significant fetal protection; an important finding given the relatively high cost of mAb medications.

These data also show mAb6B5 protection against fetal mortality occurred in the absence of significant protection against PCP-induced hemodynamic/locomotor effects (Fig. 4; Table 1). Thus maternal pharmacological effects from PCP may not reliably predict the severity of

fetal toxicity. The absence of significant differences between maternal and pup weights between treatment groups (Fig. 2) demonstrated that mAb6B5 administration did not substantially affect general health. However, there are limitations of our findings due to the small sample size and the use of a rodent model that does not mimic all aspects of a human pregnancy.

The apparent disparity between physiologic and neurologic/fetotoxic effects of PCP on mortality and morbidity are consistent with previous studies of PCP [20, 29, 36]. Notably, the marked cardiovascular and locomotor responses to PCP in our pregnant rats were consistent with PCP treatment responses in adult male rat studies [34]. Because we attempted to use a small dose of mAb binding sites to treat significantly larger molar excesses of PCP, we did not find significant reductions in maternal hemodynamic effects of PCP (Fig. 4). However, we think a larger mAb6B5 dose with larger treatment groups (e.g., n=12) could have shown mAb6B5 reductions in HD and locomotor effects without adverse mAb-related side effects. Indeed, power analysis of these data predict that groups of n=10–12 would be needed to find statistically significant results at the current doses of mAb and PCP.

In spite of the limitations of these studies, the significant decrease in the time-to-peak activity in mAb6B5-treated dams in the second trimester represents an important demonstration of maternal protection against PCP effects (Fig. 5). These data suggest anti-PCP mAb6B5 lessened PCP-induced impairment of mobility, which rendered all rats unable to ambulate in the first minutes after dosing. We also visibly observed that mAb6B5-treated animals were less affected and recovered more quickly from PCP-induced effects.

While we do not know the underlying mechanism for the improved pregnancy outcomes in mAb6B5-treated rats (Figure 3), Ikonomidou *et al.* [37] report that N-methyl-D-aspartate (NMDA) glutamate signaling is crucial for the neurodevelopment of rats (and presumably humans). In their studies, which examined neurodevelopment from GD17 through post-partum day (PD) 21, late fetal and early neonatal rats were treated with the PCP-like NMDA antagonist, MK801. Their results show that MK801 causes the most significant apoptotic neurodegeneration in fetal rats on PD7, but that significant neuroapoptosis also occurs on GD21 [37]. Jebelli *et al.* also report PCP-mediated neuroapoptosis in the 2nd trimester in rats [25]. Thus, these previous findings of NMDA antagonist-mediated fetal neurotoxicity in rats could provide an explanation for the much higher rate of fetal deaths in our vehicle PCP-treated dams. Importantly, the neurological development in the rat between GD21~PD21 corresponds to the 3rd trimester of pregnancy in humans, suggesting that human fetal vulnerability to NMDA-antagonist insult could span a significant time period during pregnancy [25, 37]. In addition, our data showing a decrease in time-to-peak activity in anti-PCP mAb6B5 dams during the 2nd trimester could suggest that mAb6B5 is indeed decreasing PCP-mediated NMDA antagonism in the developing fetal brain during a critical developmental period. While corroborative human data are scarce, case reports suggest humans do use NMDA antagonist like PCP and ketamine during pregnancy and use or abuse of either substance could potentially result in adverse neurologic effects in human infants [22, 24, 37, 38]. Additional work is needed to better delineate and verify the mechanisms of anti-PCP mAb6B5-mediated fetal protection from PCP.

Conclusion

The current studies demonstrate that anti-PCP mAb6B5 treatment of pregnant rats does not adversely affect maternal weight gain, pup weights at birth, pregnancy outcome, or fetal growth. While PCP-induced locomotor effects were only partially decreased by mAb6B5 treatment in the second trimester, mAb6B5 treatment significantly and substantively protected against fetal death due to repeated, acute PCP administration. These results

demonstrate that mAb treatment can potentially protect pregnant mothers and their offspring from adverse drug effects. Further studies with a range of mAb6B5 doses and varied PCP regimens with larger sample sizes are warranted and would likely provide important additional information regarding this new, mAb-based treatment for drug abuse in an underserved and vulnerable population: pregnant mothers and their unborn offspring.

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Highlights

- Anti-Phencyclidine (PCP) mAb6B5 reduced fetal loss in PCP-treated rats
- Anti-PCP mAb6B5 reduced PCP-induced locomotor defects in the 2nd trimester in rats
- Anti-PCP mAb6B5 did not harm pregnant rats or their offspring

Figure 1.

Study design for *in vivo* anti-PCP mAb6B5 treatment of PCP binge use in pregnant rats. MAb6B5 (iv, 45 mg/kg) was administered once per mAb6B5 half-life (↓). MAb6B5 half-life is different in the 2nd and 3rd trimester (3 days and 1 day, respectively) [30]. PCP (iv, 1 mg/kg) was administered as indicated (*).

Figure 2.

Changes in maternal rat weight during pregnancy, and pup weight at delivery.

Upper Graph. Average percentage change in maternal weight ($\pm$ SD) from the GD6 baseline weight vs. gestation day. The arrows ($\blacktriangledown$) indicate times of PCP administration.

Lower graph. Average fetal weight by litter on GD21 (mean $\pm$ SD) is shown for vehicle- (hollow bar) and mAb6B5-treated (solid bar) groups. There was no significant ($p=0.55$) difference between group mean pup weights.

Figure 3.

Pregnancy outcomes (normal versus dead pups) differed significantly (* $p=0.008$) between mAb6B5-treated dams and vehicle-treated control dams at time of delivery by cesarean section on GD22. All rats received identical treatment with PCP. MAb6B5-treated pregnant rats had significantly fewer *in utero* fetal deaths ($n=8$) than vehicle-treated controls ($n=23$). The values shown express the number of normal pups (open bar) and of fetuses/pups that suffered *in utero* fetal demise (IUFD; dead/resorbed pups, solid bars).

Figure 4.

Effects of PCP dosing on hemodynamic response in dams with vehicle or mAb6B5 treatment vs. gestation day. Data are shown as the total area under the percent change in hemodynamic response-time curve. Symbols represent individual animals in each group, and lines show group means over time. Triangles represent baseline PCP response on GD7 (before any animal had received vehicle or mAb6B5). Hollow symbols and dashes represent vehicle-treated controls, and solid symbols and lines represent mAb6B5-treated animals. Circles represent PCP response while receiving vehicle or mAb6B5 therapy.

Figure 5.

Effects of vehicle and anti-PCP mAb6B5 treatment on PCP-induced locomotor response.

Upper graph. The change in the time from PCP dosing to peak locomotor activity (Δ min).

* $p < 0.05$ compared to vehicle treated animals.

Lower graph. Area under the locomotor activity-time curve for each study day. The PCP dose on GD7 established baseline response to PCP, and vehicle and mAb6B5 treatment began on GD8, which established baseline effects of therapy. Animals receiving vehicle or mAb6B5 are shown by hollow and solid bars, respectively.

Table 1

Area under the curve for locomotor and HD parameters. (mean SD; BP=blood pressure, GD=gestation day)

GD	Locomotor Activity		Diastolic BP		Systolic BP		Pulse Pressure		Heart Rate	
	Vehicle	mAb6B5	Vehicle	mAb6B5	Vehicle	mAb6B5	Vehicle	mAb6B5	Vehicle	mAb6B5
7	1631 ± 819	1738 ± 919	1325 ± 368	1071 ± 237	1210 ± 353	1337 ± 404	1252 ± 502	1576 ± 975	149 ± 276	32 ± 189
9	1611 ± 1020	1635 ± 614	1102 ± 161	1681 ± 209	1603 ± 162	1050 ± 123	1086 ± 191	1400 ± 141	-8 ± 569	230 ± 326
12	1494 ± 1122	1624 ± 437	1195 ± 263	1422 ± 457	1434 ± 400	1226 ± 402	769 ± 451	1470 ± 616	190 ± 434	317 ± 509
18	1050 ± 548	1228 ± 492	824 ± 586	1270 ± 289	1147 ± 534	882 ± 395	1017 ± 162	905 ± 1266	-129 ± 170	592 ± 975
21	885 ± 431	854 ± 468	1317 ± 175	1334 ± 440	1537 ± 459	1522 ± 172	2041 ± 149	2002 ± 731	367 ± 358	236 ± 161