The Fate and Function of Therapeutic Anti-addiction Monoclonal Antibodies across the Reproductive Cycle of Rats

Hubbard, Jonathan J., †Laurenzana, Elizabeth M., Williams, D. Keith, Gentry, W. Brooks, Owens, S. Michael

Department of Pharmacology and Toxicology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, 72205 (JJH, EML, WBG, SMO)

Department of Biostatistics, College of Public Health, University of Arkansas for Medical Sciences, Little Rock, AR, 72205 (DKW)

Department of Anesthesiology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, 72205 (WBG)
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Corresponding Author: S. Michael Owens, Department of Pharmacology and Toxicology, 4301 W. Markham St #611, Little Rock, AR 72205. mowens@uams.edu (email). Phone 501-686-5487, fax 501-526-4618.

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Symbols and Abbreviations: AUC, area under the concentration-time curve; Cmax, maximum concentration; Cmin, minimum concentration; CIs, systemic clearance; DL, loading dose; Dm, maintenance dose; FcRn, fragment crystalizable neonatal receptor; GD, gestation day; IgG, immunoglobulin gamma; IV, intravenous; LC-MS/MS, liquid chromatography with tandem mass spectrometric detection; METH, (+)-methamphetamine; mAb, monoclonal antibody; mAb6B5 an anti-PCP mAb; mAb6H4, an anti-METH mAb; NPF, non-pregnant female; NRS, normal rat serum; PCP-HCl, phencyclidine hydrochloride; PCP, phencyclidine (free base); PK, pharmacokinetic(s); PPD, postpartum day; S.D., standard deviation; t1/2, terminal elimination half-life; tz, terminal elimination rate constant; Vd, volume of distribution.
Abstract

During preclinical development of neuroprotective anti-addiction therapeutic monoclonal antibodies (mAbs) against phencyclidine (PCP) and (+)-methamphetamine, we discovered novel, gestation stage-specific changes in mAb disposition spanning the entire reproductive cycle of female rats. Each pharmacological change was independent of mAb dose and antigen target but was precisely coincident with transitions between the gestational trimesters, parturition, and lactation periods of the female reproductive cycle. While anti-PCP mAb6B5 $t_{1/2\lambda}$ in non-pregnant females was $6.6 \pm 1.6$ days, mAb6B5 $t_{1/2\lambda}$ significantly changed to $3.7 \pm 0.4$ days, then $1.4 \pm 0.1$ days, then $3.0 \pm 0.4$ days in the second trimester, third trimester and postpartum periods, respectively ($p<0.05$ for each change). Initially, these evolving changes in mAb6B5 clearance (3.3-fold), distribution volume (1.8-fold), and elimination half-life (4.7-fold) affected our ability to sustain sufficient mAb6B5 levels to sequester PCP in the blood steam. However, understanding the mechanism(s) underlying each transition allowed development of an adaptive mAb-dosing paradigm, which substantially reduced PCP levels in dam brains and fetuses throughout pregnancy. These mAb functional studies also revealed that anti-drug mAbs readily cross the placenta prior to syncytiotrophoblast barrier maturation, demonstrating the dynamic nature of mAb pharmacokinetics in pregnancy and the importance of maintaining maternal mAb levels. These studies provide the first preclinical pregnancy model in any species for chronic mAb dosing, and could have important implications for the use of antibody therapies involving blood organ barriers (like addiction) or other chronic diseases in women of childbearing age (e.g., irritable bowel diseases, multiple sclerosis, breast cancer, rheumatoid arthritis).
Introduction

The clinical development, validation and use of evidence-based medication regimens in pregnant women present daunting challenges. Researchers need better preclinical pregnancy models for testing medication safety and efficacy, and clinicians must balance maternal and fetal safety against the need for successful treatments (Mir et al., 2007). Overcoming these hurdles requires an understanding of how gestation-dependent physiological changes drive medication disposition within a rapidly developing maternal and fetal environment. The need to understand these principles for monoclonal antibody (mAb) medications and the role of mAb blood-organ barriers like the brain and placenta presents a compelling motivation and context for the current studies (Mir et al., 2007; Kane and Acquah, 2009; Ostensen and Forger, 2009).

Current theory regarding the fate and effects of immunoglobulin G (IgG) in non-pregnant adults describes a system in which IgG molecules are salvaged from an unsaturable catabolic mechanism by a saturable “protection receptor,” which also mediates mother-to-young IgG transport (Brambell et al., 1964; Lobo et al., 2004). Multiple studies in animal models and in humans have strengthened this theory, demonstrating that the neonatal Fc receptor (FcRn) is the IgG “protection receptor” (Junghans and Anderson, 1996; Firan et al., 2001; Wani et al., 2006).

Studies in humans and rats have shown that IgG pharmacokinetics (PK) are similar and dose-independent in males and non-pregnant females with a terminal elimination half-life \( t_{1/2, z} \) of ~18-21 day (humans) and ~7-10 days (rats) (Arizono et al., 1994; Bazin-Redureau et al., 1997; Bichler et al., 2004). While mAb PK parameters in humans (and rats) appear sex-independent for males and non-pregnant females, data to support this hypothesis are limited for many species, and a clear description of the time course of the fate and function of mAbs in pregnant and lactating animals remains absent from the literature. One study in near-term pregnant rats suggests that mAb elimination increases about two-fold (Ishii et al., 1994). Data
from a small study of IgG in humans (n=6) indicates that IgG $t_{1/2,\lambda}$ might decrease during human pregnancy ($t_{1/2,\lambda}$ range~11-22 days) (Bichler et al., 2003). These studies suggest the need for further clinical and preclinical study of IgG/mAb PK. While all preclinical models have limitations, pregnant rats can model many aspects of human hemodynamic and maternofetal (e.g., uteroplacental) physiology (Dowell and Kauer, 1997; de Rijk et al., 2002), and could provide a useful preclinical model for initial safety/efficacy studies of mAb medications.

The use of antibodies against small molecular weight drugs of abuse like phencyclidine (PCP) and (+)-methamphetamine (METH) (243 and 149 g/mol, respectively) can allow the simultaneous study of \textit{in vivo} mAb fate and function in several ways. MAb-drug binding does not affect mAb disposition (\textit{i.e.}, mAb fate) (Laurenzana et al., 2003a). In addition, mAb-induced changes in the disposition of the target drug of abuse can directly reflect mAb function in mother and fetus (Riviere et al., 2000; Laurenzana et al., 2003b). Another advantage is that anti-PCP and anti-METH mAbs do not appear to cross blood-organ barriers like the brain and testis to any great extent, but they do effectively remove PCP and METH from these organs (Proksch et al., 2000; Laurenzana et al., 2003a). This redistribution of target drug out of brain tissue is the mechanism of action for the neuroprotection mediated by these anti-addiction mAb medications (Laurenzana et al., 2003b; Pentel et al., 2006). These findings suggest the potential for similar effects at the maternal blood-brain barrier, fetal blood-brain barrier and blood-placental barrier, as suggested by studies in near-term, nicotine-treated pregnant rats (Keyler et al., 2005a; Keyler et al., 2005b).

For the current studies, we used two prototype anti-small molecule (PCP and METH) murine IgG$_1$ mAb medications (anti-PCP and anti-METH) to investigate the hypothesis that mAb PKs change dramatically across the reproductive cycle of adult female rats, but that maternally administered anti-drug mAb can protect by decreasing maternal and fetal drug (\textit{e.g.}, PCP) levels. Our studies found that (1) mAb disposition dramatically and progressively changed in pregnant and lactating rats in a gestation-stage dependent manner and (2) anti-drug mAb
treatment offered therapeutic protection by decreasing maternal and fetal tissue PCP levels. The current studies provide unique insights and reveal novel mechanisms of mAb disposition and function throughout a crucial period of adult female life—the reproductive cycle.
Methods

Materials, drugs and reagents. 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), [1-(1-(phenyl-3H)cyclohexyl) piperidine] (PCP-d3), from Sigma/Isotec (Miamisburg, OH), and N-succinimidyl[2,3-3H]propionate ([3H]-NSP) from GE Life Sciences (formerly Amersham; Buckinghamshire, UK). Normal rat serum (NRS) was from Pel-Freez Biologicals (Rogers, AR). All other reagents were obtained from Fisher Scientific Co. (Fairlawn, NJ), unless otherwise specified. Water for all buffers was purified with a Milli-Q Synthesis A10 System (Millipore, Bedford, MA).

Monoclonal antibodies. Murine mAbs (IgG1 isotype, \(\kappa\) light chain) were produced as previously described (Hardin et al., 1998; Laurenzana et al., 2003a) using Wave Bioreactor (GE Healthcare, Piscataway, NJ) production followed by protein G affinity chromatography purification, and dialysis into appropriate administration vehicle (Peterson et al., 2008).

Animals. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health and with prior approval from the Institutional Animal Care and Use Committee at UAMS. Charles River Laboratories (Raleigh, NC) supplied study animals (Sprague-Dawley rats 225-250 g) and performed all catheterization and impregnation procedures. Rats were fed/watered either ad libidum (pregnant rats) or by weight-maintenance regimen (NPF), and animals were observed and weighed daily. Blood sampling was minimized and previously found not to affect hematocrit, animal health or pregnancy outcome (White et al., 2009).

MAb pharmacokinetics study design and analysis. The PKs of mAbs (IgG1 isotype, \(\kappa\)-light chain) were determined in female rats during the second (n=4) and third (n=5) pregnancy trimesters and postpartum/lactation periods (n=3). Non-pregnant female rats (n=4) served as baseline/control animals for pregnancy PK studies. Studies of PK specificity- and dose-
dependency involved 15 mg/kg of anti-METH mAb6H4 (also IgG1 with κ-light chain) and 90 mg/kg of mAb6B5, respectively (n=4). MAb PK studies were determined from mAb concentrations in serum samples collected at times designed to allow model-independent analysis of mAb distribution and elimination (Table 1). Rats in PK studies were administered a single, intravenous (IV) dose of mAb, including a trace amount of [3H]-labeled mAb, prepared as described previously (McClurkan et al., 1993). We collected serum after centrifugation of coagulated blood samples and then analyzed serum for intact mAb by size exclusion high performance liquid chromatography, and liquid scintillation spectrophotometry as previously described (Peterson et al., 2008). Fetal blood was collected on GD21 using capillary action to draw blood into 1 ml heparinized Carroway tubes after decapitation. PK analyses were performed with WinNonlin software (Pharsight, Mountain View, CA) using model-independent methods. Area under the concentration-time curve (AUC) was determined using the linear trapezoidal rule, linear interpolation and uniform weighting. All PK analyses were performed with concentration-time data from >80% of each terminal elimination phase, maximizing the accuracy of parameter calculations.

**MAb6B5 functional studies.** All *in vivo* mAb6B5 functional studies were performed using constant, subcutaneous infusion of PCP-HCl (10 mg/kg/day of free-base PCP) as previously described. The first study examined the effect of gestation stage-specific mAb PK changes on serum-binding function; animals (n=4) were administered mAb6B5 once every three days through the second and third trimesters (*i.e.*, “control” regimen). The second study (n=4) used an adaptive mAb6B5 steady-state dosing regimen and measured tissue protection and serum PCP-binding function; an IV mAb6B5 maintenance dose (D_m) was given once per mAb t_{1/2, \lambda} (second trimester: every 72 hours; third trimester: every 24 hours; *i.e.*, “adaptive” regimen). The initial mAb dose in each study was an IV loading dose (D_L) calculated by standard methods (Rowland and Tozer, 1995; Proksch et al., 2000); D_L/D_m= 30/15 mg/kg and 90/45 mg/kg of
mAb6B5 for the serum and tissue studies, respectively. Each mAb6B5 dose was prepared based on the rat weight on the day of PCP infusion pump implantation.

To examine mAb6B5 protection of dams and fetuses, a third study (n=4 per time point) was performed in which the constant PCP infusion was ~4-fold mole-equivalents higher than the mAb6B5 regimen, which was dosed per the “adaptive” schedule. In the early stages of pregnancy and fetal development, we measured mAb function at the blood-placenta barrier by monitoring changes in PCP concentration in the maternal serum whole fetal homogenates. In later stages of pregnancy, when the fetal vasculature and brain were sufficiently developed to allow proper sampling, we measured PCP levels in the fetal serum and brain.

**Tissue sampling and handling.** The blood-sampling schedule was designed to allow the assessment of IV mAb administration effects on tissue PCP levels. Samples were collected to measure minimum and maximum PCP concentrations (C\text{min} and C\text{max}, respectively) before and after each D\text{m} administration. Accordingly, 0.2 ml blood samples for both mAb functional studies were collected on GD8 before and 0.5, 1, 4, 8 and 24 h following the D\text{L} administration. For the 15 mg/kg serum mAb functional study, blood samples were also collected before and 0.5 hour following each mAb6B5 D\text{m}. For the 45 mg/kg mAb6B5 in vivo function study, maternal brain and serum and fetal tissues were collected prior to and at 0.5 and 8 h following each mAb6B5 D\text{m}. Serum was obtained from blood samples as above. Dam and fetal tissue samples were obtained after euthanasia by decapitation under isoflurane (2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane) anesthesia. Fetuses were removed by cesarean section following dam euthanasia. Pooled tissue samples (whole fetus before GD21; fetal brains on GD21) were weighed and flash-frozen immediately in liquid nitrogen. All maternal brain and fetal tissues were stored at -80°C until analyzed. The fetal blood collection method was described previously (White et al., 2009).

**Mass spectrometric detection of PCP tissue concentrations.** Serum samples were prepared as described above and stored at -20°C until analyzed. PCP was extracted from
serum by liquid-phase extraction, using a simple trichloroacetic acid treatment as previously described for METH (Hendrickson et al., 2006). PCP concentrations (ng/ml) in serum samples were determined by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS), as previously described (Hendrickson et al., 2005).

Frozen fetal tissues were thawed, diluted with four volumes of Milli-Q (MQ) water (i.e., 4 ml H$_2$O per gram of tissue) and well-homogenized using a PowerGen tissue homogenizer and stored at -80°C until analysis. PCP was extracted from thawed tissue homogenates by solid phase extraction as described previously (Hendrickson et al., 2005). Calibration standards (1, 5, 10, 50, 100, 500 and 1000 ng/ml PCP in NRS), quality control standards for serum samples (2, 80 and 800 ng/ml PCP in NRS) and quality control standards for brain samples (100 and 400 ng/ml PCP in brain homogenate from untreated male rats) were treated identically to experimental samples. LC-MS/MS procedures were performed using a Pursuit C8 100 x 2.0 mm liquid chromatography column (Varian Inc., Lake Forest, CA) and Quattro LC triple-quadrupole mass spectrometer with a Z-Spray ion interface and a Mark II source (Waters Corp., Milford, MA) as previously described (Hendrickson et al., 2005). Calibration and control sample intra-day/inter-day variability were less than 20% at the upper and lower limits of quantitation and ~6% at common sample concentrations (Hendrickson et al., 2005). Brain tissue PCP concentrations were corrected for blood PCP content as described (Laurenzana et al., 2003a).

**In vitro serum PCP binding.** Protein PCP-binding in GD21 serum samples from vehicle or mAb6B5-treated pregnant rats infused with 10 mg/kg/day PCP was measured by equilibrium dialysis against phosphate buffer containing of [$^3$H]-PCP as described (Proksch et al., 2000), using an HTDialysis cassette and dialysis strips with molecular weight cutoff of 6,000-8,000 Daltons (HT Dialysis, LLC, Gales Ferry, CT, cat. no. 1103). Quantification of [$^3$H]-PCP in both sides of each dialysis cell was performed in duplicate by liquid scintillation spectrophotometry.

**Statistical analysis.** Analysis of sample means for statistical difference between three or more groups was by one- or two-way analysis of variance followed by Tukey’s all pair wise
comparison or Bonferroni’s post-hoc analysis, respectively, after verifying that the data met all necessary statistical criteria (e.g., Gaussian distribution, etc.). MAb Vd data for NPF through the third trimester were also tested by post-hoc analysis for linear trend after ANOVA. Given the dynamic nature of maternal, placental and fetal physiology/development in pregnancy, one-way ANOVA procedures were performed with intra-day values only. PCP levels in fetal and maternal serum on GD21 were compared by two-way ANOVA for treatment and time within dam vs. pup with Bonferoni’s post-hoc analysis. The categorical data of pregnancy outcome (fetal survival) were tested for significance using Fisher’s exact test. Best-fit lines from non-linear semilog regression analysis of PCP $C_{\text{min}}$ data were tested for differences in slope by extra sum of squares F test. The D’Agostino-Pearson test verified a normal distribution for the data points used in the regression analysis ($p<0.05$ signifying non-normal distribution). All statistical analyses were performed using GraphPad Prism version 5.0 for Mac, GraphPad Software, San Diego, CA.
Results

Serum mAb pharmacokinetics in female rats. To investigate our initial hypothesis, we studied the PK of anti-PCP mAb6B5 (isotype: IgG1 with κ-light chain, PCP $K_D = 1 \text{nM}$) in adult non-pregnant, pregnant, and postpartum/lactating rats. (All data presented as $a \pm b$ represent ‘mean ± S.D.’). Compared to non-pregnant rats, the dispositional profile of mAb in pregnant rats (normal gestation: 22-23 days; ~7 days per trimester) from the second trimester through postpartum day (PPD) 21 was characterized by multiple, distinct changes in mAb $t_{1/2,z}$, clearance (Cls) and volume of distribution (Vd) (Table 2, Fig. 1). Model-independent PK analysis of mAb serum concentration-time data from female rats administered anti-PCP mAb6B5 on gestation day (GD) 8, GD15 or PPD1 found that mAb6B5 $t_{1/2,z}$ significantly changed ($p<0.05$) from pre-pregnancy through the second trimester, third trimester and lactation (Table 2).

Further analysis showed that these $t_{1/2,z}$ differences resulted from changes in Vd and Cls. MAAb6B5 Vd declined steadily throughout pregnancy ($p=0.008$ by post-hoc linear trend analysis). In contrast, Cls increased ~1.5-fold, and ~3-fold in the second and third trimesters, respectively. Following parturition, Cls was unchanged relative to the third trimester, while Vd dramatically increased ~1.8-fold (Table 2). This Vd increase produced a ~2-fold increase in mAb6B5 $t_{1/2,z}$ following parturition. Within 24-48 h after lactation ceased, the mAb terminal elimination rate slowed even further, but mAb $t_{1/2,z}$. Cls and Vd were not determined past PPD17 due to lack of data. However, mAb $t_{1/2,z}$ after lactation stopped (~GD15-17) appeared to trend toward the values for non-pregnant rats (Fig. 1).

Importantly, intact mAb was not detected in fetal serum on GD21 by high performance liquid chromatography. However, in later functional experiments with PCP (see below), there was more protein PCP-binding ($p<0.001$) in GD21 serum from mAb6B5-treated fetuses versus controls (percent bound: ~50-56 ± 1.8-3.4% vs. 34 ± 2.7%, respectively).
MAb pharmacokinetic are dose- and specificity-independent. These experiments compared the PK of low- and high-dose mAb6B5 (15 and 90 mg/kg) and 15 mg/kg anti-METH mAb6H4 (also IgG1, \(\kappa\)-light chain). The resulting data produced parallel mAb terminal elimination phases for all periods and mAbs (Fig. 2), and the area under the serum concentration-time curves in these studies from time zero extrapolated to infinity (\(AUC_0^{\infty}\)) and normalized for mAb dose were not different (\(AUC_0^{\infty}/\text{dose} = 0.07 \pm 0.01 \text{ vs. } 0.08 \pm 0.01 \text{ vs. } 0.07 \pm 0.01 \mu g*\text{day/ml}/\mu g\), respectively; \(p=0.31\)). Hematocrit values were not different between study groups and untreated pregnant rats, verifying that our minimal blood sampling did not alter either maternal health or mAb PK.

**Summary of mAb pharmacokinetic results.** The mAb PK parameters of Cls, Vd and \(t_{1/2\lambda z}\) underwent significant and substantive changes over the approximate two-month time period of the female rat’s reproductive cycle. This time period was equivalent to an approximately 12-24 month period for humans that includes pregnancy, delivery and maternal lactation. These intriguing changes led us to investigate mAb-induced changes in PCP (the target ligand for mAb6B5 binding) disposition as a measure of mAb6B5 function in the maternal-fetal unit throughout the reproductive cycle.

**In vivo mAb6B5 function during pregnancy.** For these studies pregnant rats received a continuous PCP infusion and mAb6B5 using a “control” (once every three days) fixed interval dosing schedule or an “adaptive” (once every mAb \(t_{1/2\lambda z}\) regimen. The concentration-time profiles of these two treatment regimens were significantly different: the best-fit line of PCP \(C_{\text{min}}\) during adaptive, \(t_{1/2\lambda z}\)-dependent dosing was had a zero slope, which significantly different (<0.0001) from the significantly \((p<0.0001)\) non-zero slope of the best-fit line of \(C_{\text{min}}\) from the fixed-interval control regimen (Fig. 3A,B). Semilog regression analysis of \(C_{\text{min}}\) values (Fig. 3C) showed that the adaptive regimen maintained stable mAb levels, which decreased maternal...
brain PCP levels by 46 ± 12% (95% CI 38.8-53.5%) at all time points vs. controls (n=4 per group), demonstrating the therapeutic relevance of our mAb PK findings (Table 2, Fig. 1).

**MAb6B5 pharmacokinetic antagonism at the blood-placenta barrier.** PCP concentrations in fetal homogenates from mAb-treated animals versus controls were increased by ~57% in GD9 litters at 8 h post-mAb6B5 dosing, were not significantly different on GD12, but were significantly decreased by 25-33% on GD15 (Fig. 4). PCP levels in control fetuses decreased dramatically between GD12-GD15, perhaps reflecting developmental changes in the placenta or fetal PCP endogenous binding sites. On GD21, fetal brain PCP concentrations were reduced by 33-40% versus controls (Fig. 4). Fetal PCP serum concentrations did not differ between mAb6B5- and vehicle-treated groups before mAb dosing, or 30 min or 8 h post-mAb6B5 dosing on GD21 (Fig. 5A). In contrast, maternal serum PCP levels increased by more than 3-fold, resulting in significantly higher maternal serum PCP levels versus fetal serum at 30 min and 8 h post-mAb dosing (Fig. 5A). In addition to these serum findings, fetal brain levels on GD21 were significantly (p<0.05) decreased. As an important measure of safety, maternal weight in mAb- and vehicle-treated rats were not different at any point during this study, declining slightly (<10%) in the 24 h after initiation of PCP infusion, but steadily increasing throughout pregnancy (total weight gain: ~40 ± 13% and ~34 ± 7%, respectively) at a rate not different from controls (~2.6-3% weight increase/day). It is also important that pregnancy outcome, as defined by the number of stillborn/resorbed fetuses, was not different between mAb6B5- and vehicle-treated groups (2.4% vs 3.8%, respectively, p=0.33).

Comparing the time course of changes in fetal tissue PCP concentrations relative to maternal mAb6B5 dosing on GD21 helped define the function of mAb6B5 within the fetus. On GD21, brain PCP levels in fetuses from mAb6B5-treated dams were significantly decreased relative to the serum PCP level before mAb6B5 dosing and 8 h post-mAb dosing, but not at 30 min post-mAb dosing (Fig. 5B), as evidenced by fetal brain-to-serum PCP concentration ratios.
This 30-min time-point was not significantly different from the other treatment group time-points and should not be interpreted as an increase. Importantly, the decrease in serum-to-brain PCP partitioning in fetuses from mAb6B5-treated dams persisted despite the similarity between serum PCP levels in fetuses from vehicle- and mAb6B5-treated dams (Fig. 5A,B). This possible paradox was explained by examining fetal serum unbound (i.e., free) PCP levels. The free PCP concentration in serum of mAb6B5-treated fetuses was significantly decreased versus controls at all times on GD21. Free PCP levels in mAb6B5-treated fetuses were reduced by 40 ± 5.4%, 33 ± 12.8%, and 27 ± 12.4% before and at 30 min and 8 h post-mAb6B5 dosing, respectively, compared to controls (Fig. 5C).
Discussion

These comprehensive studies provide novel insights into the fate and function of anti-addiction mAb medications across the reproductive life span of female rats. Intriguing data discovered during preclinical rat testing of a murine anti-addiction mAb (IgG₁ isotype) against PCP led us to hypothesize that the mAb PK, or “fate,” of this protein change dramatically across the span of the reproductive cycle of adult female rats, but that maternally administered anti-drug mAb can “function” to decrease target drug levels in mother and fetus. Our subsequent experiments discovered novel, dynamic PK processes governing mAb disposition and elimination in pregnant rats that spanned the antegravid, gravid and puerperal/lactation periods of the female reproductive cycle (Fig. 1). However, understanding the pharmacological characteristics of each PK change allowed the design of an adaptive dosing paradigm, which maintained the mAb6B5-mediated maternal-fetal protection from PCP throughout pregnancy (Fig. 3-4).

These data demonstrate evolving changes in mAb $t_{1/2\lambda z}$, Cls and Vd during pregnancy (Fig. 1) that are best understood by the PK relationships, $t_{1/2\lambda z} = 0.7 \times Vd/Cls$. Among these variables, changes in $t_{1/2\lambda z}$ depend on the independent PK processes of Cls and Vd. To our knowledge, there is no other example of a medication that exhibits multiple discrete PK transition periods (Table 2, Fig.1), which are all linear and dose-independent (Fig. 2) within each phase. Furthermore, no systematic investigation of IgG PK in pregnant humans or non-human primates is available in the literature, and there is no clear evidence supporting or contradicting the applicability of our findings to human patients. However, human studies suggest that IgG levels (Benster and Wood, 1970), and mAb $t_{1/2\lambda z}$ may decrease in normal human pregnancy (Bichler et al., 2003).

Our data clearly shows that normal biological developmental processes during a healthy rat pregnancy can produce significant changes in mAb PKs and pharmacodynamics. Previous
studies in non-pregnant animals report IgG/mAb clearance results from a complex set of physiological and cellular processes, with the FcRn salvage pathway playing a central role (Israel et al., 1996; Junghans and Anderson, 1996; Wani et al., 2006). While we hypothesize the gestation-stage dependent mAb increases and decreases in Cls in our studies would likely have some correlation with FcRn expression and functional changes during rat pregnancy, the current literature lacks explicit evidence for or against this hypothesis.

However, the mAb Vd changes we observed (Table 2) suggest that FcRn expression/functional changes are not solely responsible for all changes in mAb disposition. Indeed, cardiovascular/hematologic changes during normal pregnancy likely also contributing. Specifically, the physiological PK simulation model of Garg and Balthasar (2007) suggests that normal plasma volume increases and blood pressure decreases in rat pregnancy (Dowell and Kauer, 1997) could lead to the IgG Vd and Cls findings in our studies (Table 2). The development of the mammary glands and deposition/accumulation of IgG in colostrum late in pregnancy (Lu et al., 2007) could also represent an extravascular sink for IgG, possibly contributing to the observed third trimester and postpartum Vd changes (Table 2). Importantly, these hemodynamic changes in the rat model could mimic human changes (Dowell and Kauer, 1997), suggesting that mAb Vd and Cls changes may also occur during human pregnancy.

While mAb-treatment consistently lowered maternal brain PCP levels, fetal PCP levels responded more dynamically (Fig. 4). The increased fetal tissue PCP concentrations on GD9 and GD12 likely reflect increase amounts of mAb-bound PCP, as tissue PCP levels directly reflect mAb levels (Laurenzana et al., 2003a; Laurenzana et al., 2003b; Pentel et al., 2006). Mother-to-fetus mAb transfer in this period is not surprising, since the rat blood-placental barrier maturation occurs between GD12-15 (de Rijk et al., 2002). The decrease in fetal PCP levels from GD12-GD15 could result from blood-placenta barrier maturation (Fig. 4), although endogenous fetal PCP-binding site stoichiometry changes could also contribute. The relatively steady levels of bound and free PCP between GD21 time points in fetuses (Fig. 5) provides
strong evidence that the placental barrier substantially blocked maternal-fetal passage of mAb in rats, consistent with previous work (Arizono et al., 1994; Nekhayeva et al., 2005). Thus, mAb appears to most efficiently antagonize PCP entry into the fetus after placental barrier maturation (Fig. 4). These functional data seem applicable to human pregnancy, as rat placental maturation models many aspects of the human process (de Rijk et al., 2002).

Issues of antenatal IgG transport are one area of weakness for our model. While we could not detect mAb in fetal serum, the antenatal trans-placental transport of IgG in humans would lead to higher fetal levels of maternal IgG and mAb (Simister, 2003). This could raise concerns about possible effects of higher mAb-associated fetal drug levels and an extended $t_{1/2, z}$ of mAb-bound drug (Lobo et al., 2004). While fetal PCP data from GD9 confirm that mAb-bound drug can increase total drug levels while the placental barrier is mAb-permeable (Fig. 4), both of these possible concerns are addressed by remembering that mAb-associated increases in total drug levels consist almost entirely of inactive, mAb-bound drug (Laurenzana et al., 2003b). Thus, the apparently extended drug $t_{1/2, z}$ in mAb-dosed animals does not reflect an extended $t_{1/2, z}$ of the active drug, but of inactive, mAb-bound drug. Even if a small amount of free drug is present due to the natural on-off process of mAb-binding, previous work has shown that not all drug must be mAb-bound for protection to occur (Laurenzana et al., 2003b; Pentel et al., 2006; Pitas et al., 2006). Our data also provide evidence regarding the mechanism of mAb antagonism of target drug disposition. We think that the lower serum unbound PCP levels in mAb6B5-treated fetuses (Fig. 5C) demonstrates that maternal mAb6B5 serum levels accounted for decreased fetal (Fig. 4) and maternal brain levels. Clearly, more work is needed to assess the risk (if any) of mAb-bound drug in the fetus, but we observed no adverse effects in any animals. The need for mAb studies in women is especially compelling given that the highest incidence of many diseases targeted by mAb medications (e.g., irritable bowel diseases, multiple sclerosis, breast cancer, rheumatoid arthritis) occurs in women of childbearing age (Mir et al., 2007; Kane and Acquah, 2009).
The mAb-mediated reductions in tissue PCP levels are an important surrogate marker for the potential therapeutic efficacy of anti-addiction medications (Fig. 4). Importantly, the mAb6B5-mediated decreases in maternal brain PCP concentrations reported here (95% CI 39-53%; range 23-63%) were greater than the 20% reduction that protected male rats from adverse PCP effects (Laurenzana et al., 2003b). These maternal brain PCP reductions levels carry potentially important implications for human treatment, since high brain levels consistently correlate with adverse effects (Kantak et al., 2000; Laurenzana et al., 2003b; Pentel et al., 2006). While it is often unclear whether fetal effects of illicit drugs (e.g., low birth weight) result directly from the drug or from drug effects on maternal health/behavior (Ali et al., 1989; Glantz and Woods, 1993), it seems reasonable that protecting a mother from the decision-impairing effects of PCP (or other illicit drugs like METH) could dramatically improve the medical and psychosocial prognoses for both the mother and her offspring.

Another crucial point from our studies relates to the effective dose and clinical feasibility: rats in these studies received 4-fold more PCP on a molar basis than anti-PCP mAb binding sites. The discovery that anti-drug mAb or IgG appears to protect despite theoretical mAb antigen binding site-saturation agrees with previous studies (Laurenzana et al., 2003b; Pentel et al., 2006; Pitas et al., 2006), and supports the clinical and economic feasibility of extended, chronic mAb dosing treatment strategies in humans (i.e., months to years).

In summary, these studies provide the most comprehensive findings so far of the mAb dispositional profile in the antegravid, gravid, puerperal and lactating females of any species, and in their offspring in utero. Our findings prompt intriguing questions: why are the PK changes so precisely timed at each gestational phase and at parturition, are these changes found in pregnant women, and could an mAb be a biomarker marking maternal blood placental barrier closure? In addition to raising these questions, these findings provide much needed (Mir et al., 2007; Kane and Acquah, 2009; Ostensen and Forger, 2009) dosing and safety guidelines for mAb therapy in preclinical pregnancy treatment models. Our report also presents a useful
animal model for studying mAb therapy for drug addiction during pregnancy. These findings demonstrate that mAb PKs changes throughout the female rat reproductive cycle in unprecedented ways and strongly suggest that (1) a mAb dosing regimen adaptive to dynamic mAb PK can be achieved and can maintain neuroprotective mAb steady-state levels throughout pregnancy, (2) both FcRn-mediated clearance and gestational hemodynamic changes appear to play independent, but interrelated, roles in gestation-stage dependent mAb PK changes, (3) blood-organ barriers in rapid equilibration with plasma are central to mAb-mediated PK antagonism of the drug of abuse in this study, and (4) maternal administration of anti-drug mAb in pregnancy appears to safely reduce maternal and fetal PCP exposure.
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Author Contributions

Participated in research design: Hubbard, Laurenzana, Owens, Gentry
Conducted Experiments: Hubbard, Laurenzana
Performed data analysis: Hubbard, Owens, Williams
Wrote or contributed to writing of the manuscript: Hubbard, Owens
Other: Owens and Hubbard secured funding for the research
References


Laurenzana EM, Byrnes-Blake KA, Milesi-Halle A, Gentry WB, Williams DK and Owens SM (2003a) Use of anti-(+)-methamphetamine monoclonal antibody to significantly alter (+)-
methamphetamine and (+)-amphetamine disposition in rats. Drug Metab Dispos 31:1320-1326.


Footnotes

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Legends for Figures

Figure 1. MAb concentration-time profile across the reproductive cycle of adult female rats

Serum mAb concentration-time data in non-pregnant rats after IV administration of 15 mg/kg of anti-PCP mAb6B5 (labeled “Antegravid Period”; n=4 with mean ± S.D.; ♦). These data represent the baseline adult values for mAb6B5 disposition. All other plots show the mAb6B5 serum concentration-time data in pregnant or postpartum rats administered mAb6B5 IV at the beginning of the second trimester (15 mg/kg ●; n=4), at the beginning of the third trimester (10 mg/kg ○; n=5), and the day of parturition (postpartum day 0; 15 mg/kg ■; n=3), respectively.

*Third trimester mAb $t_{1/2\lambda z}$ values determined in pregnant rats dosed on either GD8 or GD15 are not statistically different from each other ($t_{1/2\lambda z} = 1.0±0.2$ and $1.4±0.1$ days, respectively). These values are significantly different (p<0.05) from mAb $t_{1/2\lambda z}$ values determined during the second trimester and postpartum periods ($t_{1/2\lambda z} = 3.7±0.3$ and $3.0±0.4$ days, respectively).

†All gestational and postpartum $t_{1/2\lambda z}$ values are significantly different (p<0.05) from NPF. ($\alpha =$ day of conception; $\downarrow =$ approximate day of implantation; the label ’~14 days’ signifies an approximation, since this $t_{1/2\lambda z}$ value appeared to be in the process of reverting back to the antegravid values and was derived from only several days of data points.)

Figure 2: MAb PK dose- and specificity-independence.

Serum anti-PCP mAb6B5 (○,●) and anti-METH mAb6H4 (□) concentration-time data (mean ± s.d.), and best-fit lines (——, - - - - -) to the WinNonlin log-linear regression fit of these data, from serum samples taken between GD8-GD21 from pregnant rats dosed IV with 15 mg/kg mAb6B5 or mAb6H4 (●,□) or 90 mg/kg mAb6B5 (○) on GD8. Data are from groups (n=4) of timed-pregnant SD rats. MAb $t_{1/2\lambda z}$ are not statistically different between any treatment groups.
Figure 3. Maintenance of therapeutic mAb levels in pregnancy

Serum PCP concentrations in rats infused with PCP and treated with two different mAb6B5 dosing strategies (n=4 per group). MAb6B5 dosing events are noted (†). Control animals (□) received administration vehicle without mAb. All rats dosed with mAb6B5 received a loading dose of mAb6B5 on GD9. Total serum PCP concentration-time profiles from rats administered (data are mean ± S.D.):

A. 15 mg/kg (■) mAb6B5 administered once every 3 days. (D’Agostino-Pearson test statistic = 2.22, p = 0.33). Blood sampling occurred before and after each mAb6B5 administration (see methods).

B. 45 mg/kg (●) mAb6B5 administered once per mAb $\frac{t_1}{2\lambda}$, based on the $\frac{t_1}{2\lambda}$ values from Table 2. (D’Agostino-Pearson test statistic = 2.09, p = 0.35). Blood sampling was limited to GD15, GD17, GD19 and GD21 to minimize blood collections (see methods).

Non-linear, semi-log regression analysis of serum PCP $C_{min}$ values from animals in each type of mAb6B5 dosing strategy are shown as dashed lines. The line slopes from the two regimens were statistically different (p<0.0001), with a ~7-fold change in best-fit line slopes (m) of $C_{min}$ between the control (m = -6.9x10^{-2} ± 0.9x10^{-2}; *p < 0.0001) and adaptive regimens (m = -1.0x10^{-2} ± 0.6x10^{-2}; p < 0.07).

Figure 4. Fetal neuroprotection by mAb6B5

PCP concentrations (mean ± S.D.) in fetuses (whole fetus ▼,▼ and fetal brain △,▲) from pregnant rats given 10 mg/kg/day of PCP by constant, subcutaneous infusion from GD8-GD21 and treated with either vehicle (empty symbol) or mAb6B5 (filled symbol) once per mAb $\frac{t_1}{2\lambda}$ (based on data from Table 2), beginning on GD9. Accordingly, each “T -5 min” data point shows the residual protective effects of the previous mAb dose and corresponds to maternal serum $C_{min}$, as in Fig. 3B, while “T 30 min” and “T 8 hr” data points show effects of the same-day mAb-
treatment. Control samples from vehicle-treated (Veh-Tx) animals were from animals dosed with vehicle only and were collected at the same time as the “T -5 min” samples. For days GD9-GD15, the PCP concentration values are from the analysis of whole fetuses due to the limited amount of brain tissue available in early pregnancy. Fetal brains were large enough on GD21 for PCP tissue quantitation. PCP levels were 25-33% and 33-40% reduced vs same-day controls on GD15 and GD21, respectively.

Figure 5. Anti-PCP mAb6B5 Pharmacokinetic antagonism of materno-fetal PCP disposition
As in for Fig. 4 data, these data are from pregnant rats given 10 mg/kg/day of PCP by constant, subcutaneous infusion from GD8-21 and treated with either vehicle (empty symbol) or mAb6B5 (filled symbol) once per mAb $t_{1/2λ}$ from GD9-GD21. The “T -5 min” data point shows the residual protective effects of the previous mAb dose (given one $t_{1/2λ}$ before GD21 dosing) and corresponds to maternal serum $C_{min}$, as in Fig. 3B, while “T 30 min” and “T 8 hr” data points show effects of the same-day mAb-treatment. Control samples from vehicle-treated (Veh-Tx) animals were collected at the same time as the “T -5 min” samples.

A. Serum PCP concentrations in dams (○,●) and their pups (▽,▼) on GD21 in rats dosed throughout pregnancy with PCP and treated with either vehicle (○,▽) or mAb6B5 (●,▼). Data points are mean ± S.D. Data points from dams at 30 min and 8 hr after mAb treatment were significantly higher than control values (*p<0.05). Symbols without a visible error bar represent means with S.D. values that are covered by the symbol.

B. Brain to serum PCP concentration ratios on GD21 in pups from vehicle- (empty bar) and mAb6B5-treated (filled bars) groups. Tissue was collected at T -5 min, T 30 min, and T 8 hr from mAb administration (*p<0.05 vs controls).

C. Bound (empty bar) and unbound, or “free,” (filled bar) PCP concentrations in fetal serum from control (i.e., vehicle-treated) and mAb6B5-treated litters on GD21. Total (bound + free)
PCP concentrations were not different between groups (p=0.39), but free PCP concentrations were significantly lower in mAb6B5-treated litters than controls (*p<0.05, **p<0.01).
Table 1

Table 1. Blood sampling schedule for mAb PK studies.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Pregnancy Phase</th>
<th>mAb day of administration</th>
<th>mAb dose</th>
<th>Blood draw days after day 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPF</td>
<td>--</td>
<td>15 mg/kg</td>
<td>Every 48 h for 27 days</td>
</tr>
<tr>
<td>2\textsuperscript{nd} Trimester</td>
<td>GD8</td>
<td>15 mg/kg</td>
<td>GD12-15: every 12 h; GD16-21: every ~36 h</td>
</tr>
<tr>
<td>3\textsuperscript{rd} Trimester</td>
<td>GD15</td>
<td>10 mg/kg</td>
<td>GD16-PPD0: every 12 h; PPD0-7: every 24 h</td>
</tr>
<tr>
<td>Postpartum</td>
<td>PPD1</td>
<td>15 mg/kg</td>
<td>PPD2-15: every 72 h; PPD16-21: every 24 h</td>
</tr>
<tr>
<td>Dose-dependence</td>
<td>GD8</td>
<td>90 mg/kg</td>
<td>GD11; GD13-16: every 12 h; GD17-21: every 24 h</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Study day 0 was defined as the day of mAb administration. Blood sampling on day 0 occurred for all groups at 0.25, 2, 4, 8, and 24 h after mAb dosing. Subsequent blood sampling occurred as indicated.
Table 2.

Pharmacokinetic parameters for anti-PCP mAb6B5 in non-pregnant (nulligravid) and pregnant female rats across their reproductive cycle.

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Nulligravid</th>
<th>2nd Trimester: GD8~16</th>
<th>3rd Trimester: ~GD15-21</th>
<th>Postpartum PPD1-15</th>
</tr>
</thead>
<tbody>
<tr>
<td>t_{1/2} (days)</td>
<td>6.6 ± 1.6</td>
<td>3.7 ± 0.3*</td>
<td>1.4 ± 0.1*†</td>
<td>3.0 ± 0.4*</td>
</tr>
<tr>
<td>Vd (ml/kg)</td>
<td>82.1 ± 15</td>
<td>70.4 ± 3‡</td>
<td>59.7 ± 8.6‡</td>
<td>106 ± 22*</td>
</tr>
<tr>
<td>Cl_s (ml/day/kg)</td>
<td>9.0 ± 2.3</td>
<td>13.1 ± 2.8‡</td>
<td>29.9 ± 4.9*</td>
<td>24.5 ± 4.8*</td>
</tr>
<tr>
<td>λ_z (day^{-1})</td>
<td>0.11 ± 0.03</td>
<td>0.19 ±</td>
<td>0.49 ±</td>
<td>0.23 ± 0.03*</td>
</tr>
</tbody>
</table>

The calculations for these final PK values was generated by non-compartmental analysis of mAb6B5 concentration-time data using uniform weighting of data. All values are mean ± standard deviation (S.D.). NPF = non-pregnant female; GD = gestation day; PPD = postpartum day; λ_z = terminal elimination rate constant; Vd = apparent volume of distribution.

*p<0.05 vs. NPF; †p<0.05 vs. 2nd Trimester; ‡p<0.05 vs. Postpartum
Figure 1

Serum mAb6B5 Conc. (µg/ml)

Time (Days)

Antebravid Period

Gestation Day

Postpartum Day

Parturition

2nd Trimester

3rd Trimester

Pups Weaned

6.6 days

3.7 days

3.0 days

1.4 days

1.0 days

3.3 days

~14 days

NPF (baseline) - 15 mg/kg

Preg (2nd Tri) - 15 mg/kg

Preg (3rd Tri) - 10 mg/kg

Postpartum - 15 mg/kg

Best fit line of ave conc.
Figure 2

Serum mAb Conc (µg/ml)

4.3 days

3.4 days

3.7 days

1.4 days

1.1 days

1.0 days

8 12 16 20

Time (Gestation Day)

Legend:
- O Anti-PCP mAb6B5 - 90 mg/kg
- ● Anti-PCP mAb6B5 - 15 mg/kg
- □ Anti-METH mAb6H4 - 15 mg/kg
Figure 3

A

Serum PCP Conc (ng/ml)

Fit of "Control Regimen" $C_{min}$

B

Serum PCP Conc (ng/ml)

Fit of "Adaptive Regimen" $C_{min}$
Figure 4

Fetal Tissue PCP Conc (ng/g)

Whole Fetus

Fetal Brain

Time (Gestation Day)

0 500 1000 1500

400 ng/g
200 ng/g

Veh-Tx T-30 min T-8 hr

Veh-Tx T-5 min T-30 min T-8 hr

Veh-Tx T-5 min T-30 min T-8 hr

Veh-Tx T-5 min T-30 min T-8 hr

Time from mAb dosing

* * *
Figure 5

A

Serum PCP Concentration (ng/ml)

B

Pup Brain:Serum PCP Ratio

Vehicle-Treated

mAb6B5-Treated

C

Fetal Serum PCP Concentration (ng/ml)

Veh-Tx

T-5 min

T-30 min

T-8 hr

Time (from mAb-treatment)