The Fate and Function of Therapeutic Antiaddiction Monoclonal Antibodies across the Reproductive Cycle of Rats

Jonathan J. Hubbard, Elizabeth M. Laurenzana, 1 D. Keith Williams, W. Brooks Gentry, and S. Michael Owens

Departments of Pharmacology and Toxicology (J.J.H., E.M.L., W.B.G., S.M.O.) and Anesthesiology (W.B.G.), College of Medicine, and Department of Biostatistics, College of Public Health (D.K.W.), University of Arkansas for Medical Sciences, Little Rock, Arkansas

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ABSTRACT

During preclinical development of neuroprotective antiaddiction 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. Whereas anti-PCP mAb6B5 terminal elimination half-life ($t_{1/2z}$) in nonpregnant females was $6.6 \pm 1.6$ days, the mAb6B5 $t_{1/2z}$ 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 bloodstream. However, understanding the mechanisms 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 antidrug mAbs readily cross the placenta before 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 (such as 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 such as the brain and placenta presents compelling motivation and context for the current studies (Mir et al., 2007; Kane and Acquah, 2009; Østensen and Forger, 2009).

ABBREVIATIONS: mAb, monoclonal antibody; $C_{\text{min}}$, minimum concentration; Cls, systemic clearance; $D_L$, loading dose; $D_m$, maintenance dose; FcRn, neonatal Fc receptor; GD, gestation day; LC-MS/MS, liquid chromatography with tandem mass spectrometric detection; METH, (+)-methamphetamine; mAb6H4, an anti-METH mAb; PCP, phencyclidine; mAb6B5 an anti-PCP mAb; NPF, nonpregnant female; NRS, normal rat serum; PK, pharmacokinetics; PPD, postpartum day; $t_{1/2z}$, terminal elimination half-life; $k_z$, terminal elimination rate constant; $V_d$, volume of distribution; ANOVA, analysis of variance; AUC, area under the serum concentration-time curves.
Current theory regarding the fate and effects of IgG in nonpregnant 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 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 nonpregnant females with a terminal elimination half-life ($t_{1/2\alpha}$) of ~18 to 21 days in humans and ~7 to 10 days in rats (Arizono et al., 1994; Bazin-Redureau et al., 1997; Bichler et al., 2004). Although mAb PK parameters in humans (and rats) seem sex-independent for males and nonpregnant 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 approximately 2-fold (Ishii et al., 1994). Data from a small study of IgG in humans ($n = 6$) indicates that IgG $t_{1/2\alpha}$ might decrease during human pregnancy ($t_{1/2\alpha}$ range ~11–22 days) (Bichler et al., 2003). These studies suggest the need for further clinical and preclinical study of IgG/mAb PK. Although 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 such as phencyclidine (PCP) and (+)-methamphetamine (METH) (243 and 149 g/mol, respectively) can allow the simultaneous study of in vivo mAb fate and function in several ways. mAb drug binding does not affect mAb disposition (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 (Riviére et al., 2000; Laurenzana et al., 2003b). Another advantage is that anti-PCP and anti-METH mAbs do not seem to cross blood-organ barriers such as the brain and testis to any great extent, but they do effectively remove PCP and METH from these organs (Bichler et al., 2003). Rats in PK studies were administered a single intravenous dose of mAb, including a trace amount of [3H]-labeled 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 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 described previously (Peterson et al., 2008). Fetal blood was collected on gestation day (GD) 21 using capillary action to draw blood into 1 ml of heparinized Cuvettes 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 by 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.

mAb Pharmacokinetics Study Design and Analysis. The PKs of mAbs (IgG isotype, κ-light chain) were determined in female rats during the second ($n = 4$) and third ($n = 5$) pregnancy trimesters and postpartum/lactation periods ($n = 3$). Nonpregnant female rats ($n = 4$) served as baseline/control animals for pregnancy PK studies. Studies of PK specificity and dose dependence involved 15 mg/kg of anti-METH mAb6B5 (also IgG1, with κ-light chain) and 90 mg/kg of mAb6B5, respectively ($n = 4$). mAb PK studies were performed with IgG1 mAbs prepared as described previously (Laurenzana et al., 2003a). Monoclonal Antibodies. Murine mAbs (IgG1, isotype, κ-light chain) were produced as described previously (Hardin et al., 1998; Laurenzana et al., 2003a) using Wave Bioreactor (GE Healthcare) production followed by protein G affinity chromatography purification and dialysis into the appropriate administration vehicle (Peter-son 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 the University of Arkansas for Medical Sciences. Charles River Laboratories Inc. (Raleigh, NC) supplied study animals (Sprague-Dawley rats, 225–250 g) and performed all catherization and impregnation procedures. Rats were fed/watered either ad libitum (pregnant rats) or by weight-maintenance regimens (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, κ-light chain) were determined in female rats during the second ($n = 4$) and third ($n = 5$) pregnancy trimesters and postpartum/lactation periods ($n = 3$). Nonpregnant female rats ($n = 4$) served as baseline/control animals for pregnancy PK studies. Studies of PK specificity and dose dependence involved 15 mg/kg of anti-METH mAb6B5 (also IgG1, with κ-light chain) and 90 mg/kg of mAb6B5, respectively ($n = 4$). mAb PK studies were performed with IgG1 mAbs 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 described previously (Peterson et al., 2008). Fetal blood was collected on gestation day (GD) 21 using capillary action to draw blood into 1 ml of heparinized Cuvettes 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 by 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.
PCP-HCl (10 mg/kg/day of free-base PCP) as described previously. 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 3 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 intravenous mAb6B5 maintenance dose (D_m) was given once per mAb t_{1/2,\text{m}} (second trimester: every 72 h; third trimester: every 24 h; i.e., "adaptive" regimen). The initial mAb dose in each study was an intravenous loading dose (D_l) calculated by standard methods (Rowland and Tozer, 1995; Proksch et al., 2000); D_l/D_m = 30/15 and 90/45 mg/kg of mAb6B5 for the serum and tissue studies, respectively. Each mAb6B5 dose was prepared based on the rat’s 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 intravenous mAb administration effects on tissue PCP levels. Samples were collected to measure minimum PCP concentration (C_{min}) and maximum PCP concentrations before and after each D_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 after the D_l administration. For the 15 mg/kg serum mAb functional study, blood samples were also collected before and 0.5 h after each mAb6B5 D_m. For the 45 mg/kg mAb6B5 in vivo function study, maternal brain and serum and fetal tissues were collected before and at 0.5 and 8 h after each mAb6B5 D_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-trifluoroethane) anesthesia. Fetuses were removed by Cesarean section after 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 described previously 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 described previously (Hendrickson et al., 2005). Frozen fetal tissues were thawed, diluted with four volumes of Milli-Q water (i.e., 4 ml of H_2O per gram of tissue), well homogenized using a tissue homogenizer (PowerGen 125; Thermo Fisher Scientific), 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 by using a Pursuit C8 100 × 2.0-μm liquid chromatography column (Varian Inc., Palo Alto, CA) and Quattro LC triple-quadrupole mass spectrometer with a Z-Spray ion interface and a Mark II source (Waters, Milford, MA) as described previously (Hendrickson et al., 2005). Calibration and control sample intraday/interday 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 previously (Laurenzana et al., 2005a).

**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 [3H]PCP as described previously (Proksch et al., 2000), using an HHT dialysis cassette and dialysis strips with molecular mass cutoff of 6000 to 8000 Da (HT Dialysis, LLC, Gales Ferry, CT). Quantification of [3H]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 pairwise comparison or Bonferroni’s post hoc analysis, respectively, after verifying that the data met all necessary statistical criteria (e.g., Gaussian distribution, etc.). mAb volume of distribution (V_m) 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 only with intraday values. PCP levels in fetal and maternal serum on GD21 were compared by two-way ANOVA for treatment and time within dam versus pup with Bonferroni’s post hoc analysis. The categorical data of pregnancy outcome (fetal survival) were tested for significance by using Fisher’s exact test. Best-fit lines from nonlinear semilog regression analysis of PCP C_{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 with Prism version 5.0 for Mac (GraphPad Software Inc., San Diego, CA).

**Results**

**Serum mAb Pharmacokinetics in Female Rats.** To investigate our initial hypothesis, we studied the PK of anti-PCP mAb6B5 (isotype: IgG_1 with κ-light chain, PCP K_{D} = 1
nM) in adult nonpregnant, pregnant, and postpartum/lactating rats (all data presented as \( \bar{a} \pm b \) represent mean ± S.D.). Compared with nonpregnant 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 \( t_{1/2\alpha} \), systemic clearance (Cls), and \( V_d \) (Table 2, Fig. 1). Model-independent PK analysis of mAb serum concentration-time data from female rats administered anti-PCP mAb6B5 on GD8, GD15, or PPD1 found that mAb6B5 \( t_{1/2 \alpha} \) significantly changed (\( p < 0.05 \)) from prepregnancy through the second trimester, third trimester, and lactation (Table 2).

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

It is noteworthy that 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 (percentage bound: ~50–56 ± 1.8–3.4% versus 34 ± 2.7%, respectively).

**mAb Pharmacokinetics Are Dose- and Antigen-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 AUC in these studies from time 0 extrapolated to infinity (AUC\(_{\infty} \)) and normalized for mAb dose were not different (AUC\(_{\infty} \)/dose = 0.07 ± 0.01 versus 0.08 ± 0.01 versus 0.07 ± 0.01 \( \mu g \times \) 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, \( V_d \), and \( t_{1/2 \alpha} \) underwent significant and substantive changes over the approximate 2-month time period of the female rat’s reproductive cycle. This time period was equivalent to an approximately 12- to 24-month period for humans that includes pregnancy, delivery, and maternal

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**TABLE 2**

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

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Nulligravid (NPF)</th>
<th>Gestation</th>
<th>Postpartum (PPD1–15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd Trimester: GD8–16</td>
<td>3rd Trimester: GD10–21</td>
<td>4th and 5th Trimester: GD15–21</td>
</tr>
<tr>
<td>( t_{1/2 \alpha} ) (days)</td>
<td>6.6 ± 1.6</td>
<td>3.7 ± 0.2*</td>
<td>4.8 ± 0.04</td>
</tr>
<tr>
<td>( V_d ) (ml/kg)</td>
<td>82.1 ± 15</td>
<td>70.4 ± 3†</td>
<td>59.7 ± 8.6†</td>
</tr>
<tr>
<td>Cls (ml/day/kg)</td>
<td>9.0 ± 2.3</td>
<td>13.1 ± 2.8‡</td>
<td>29.9 ± 4.9‡</td>
</tr>
<tr>
<td>( \lambda_\alpha ) (day(^{-1}))</td>
<td>0.11 ± 0.03</td>
<td>0.19 ± 0.02</td>
<td>0.49 ± 0.07‡</td>
</tr>
</tbody>
</table>

* \( p < 0.05 \) vs. NPF; † \( p < 0.05 \) vs. 2nd trimester; ‡ \( p < 0.05 \) vs. postpartum.

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**Fig. 1.** mAb concentration-time profile across the reproductive cycle of adult female rats. Serum mAb concentration-time data in nonpregnant rats after intravenous 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 intravenously 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). **Best fit line of ave conc.** It is noteworthy that 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 (percentage bound: 50–56 ± 1.8–3.4% versus 34 ± 2.7%, respectively). **mAb Pharmacokinetics Are Dose- and Antigen-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 AUC in these studies from time 0 extrapolated to infinity (AUC\(_{\infty} \)) and normalized for mAb dose were not different (AUC\(_{\infty} \)/dose = 0.07 ± 0.01 versus 0.08 ± 0.01 versus 0.07 ± 0.01 \( \mu g \times \) 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, \( V_d \), and \( t_{1/2 \alpha} \) underwent significant and substantive changes over the approximate 2-month time period of the female rat’s reproductive cycle. This time period was equivalent to an approximately 12- to 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 3 days) fixed-interval dosing schedule or an “adaptive” (once every mAb $t_{1/2,z}$) regimen. The concentration-time profiles of these two treatment regimens were significantly different: the best-fit line of PCP $C_{min}$ during adaptive, $t_{1/2,z,c}$-dependent dosing had a zero slope, which was significantly different ($<0.0001$) from the significantly ($p < 0.0001$) nonzero slope of the best-fit line of $C_{min}$ from the fixed-interval control regimen (Fig. 3A).

Semilog regression analysis of $C_{min}$ values (Fig. 3B) showed that the adaptive regimen maintained stable mAb levels, which decreased maternal brain PCP levels by 46% versus 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 $\sim$57% in GD9 litters at 8 h after mAb6B5 dosing, were not significantly different on GD12, but were significantly decreased by 25 to 33% on GD15 (Fig. 4). PCP levels in control fetuses decreased dramatically between GD12 and GD15, perhaps reflecting developmental changes in the placenta or fetal PCP endogenous binding sites. On GD21, fetal brain PCP concentrations were reduced by 33 to 40% versus controls (Fig. 4). Fetal PCP serum concentrations did not differ between mAb6B5- and vehicle-treated groups before mAb dosing or 30 min or 8 h after mAb6B5 dosing on GD21 (Fig. 5A). In contrast, maternal serum PCP levels increased by more than 3-fold, resulting in significantly higher maternal serum PCP levels versus fetal serum at 30 min and 8 h after mAb dosing (Fig. 5A). In addition to these serum findings, fetal brain levels on GD21 were significantly ($p < 0.05$) decreased. As an important measure of safety, maternal weights in mAb- and vehicle-treated rats were not different at any point during this study, declining slightly ($<10\%$) in the 24 h after initiation of PCP infusion, but steadily increasing throughout pregnancy (total weight gain: $\sim40 \pm 13$ and $\sim34 \pm 7\%$, respectively) at a rate not different from controls ($\sim2.6–3\%$ 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 versus 3.8%, respectively, $p = 0.33$).

Comparing the time course of changes in fetal tissue 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.) are shown. 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 Materials and Methods). B, 45 mg/kg (●) mAb6B5 administered once per mAb $t_{1/2,z}$, based on the $t_{1/2,z}$ 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 Materials and Methods). Nonlinear, semilog 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 $\sim7$-fold change in best-fit line slopes ($m$ of $C_{min}$ between the control ($m = -6.9 \times 10^{-2} \pm 0.9 \times 10^{-2}$; $*, p < 0.0001$) and adaptive regimens ($m = -10 \times 10^{-2} \pm 0.6 \times 10^{-2}$; $p < 0.07$).
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 after mAb dosing, but not at 30 min after 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. It is noteworthy that 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. 5, A and 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 after mAb6B5 dosing, respectively, compared with controls (Fig. 5C).

**Discussion**

These comprehensive studies provide novel insights into the fate and function of antiaddiction mAb medications across the reproductive life span of female rats. Intriguing data discovered during preclinical rat testing of a murine antiaddiction mAb (IgG1 isotype) against PCP led us to hypothesize that the mAb PK, or “fate,” of this protein changes dramatically across the span of the reproductive cycle of adult female rats, but that maternally administered antidrug mAbs 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 (Figs. 3 and 4).

These data demonstrate evolving changes in mAb $t_{1/2,az}$, Cls, and $V_d$ during pregnancy (Fig. 1) that are best understood by the PK relationships, $t_{1/2,az} = 0.7 \times V_d$/Cls. Among these variables, changes in $t_{1/2,az}$ depend on the independent PK processes of Cls and $V_d$. 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 nonhuman 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 $t_{1/2,az}$ may decrease in normal human pregnancy (Bichler et al., 2003).

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

However, the mAb $V_d$ changes we observed (Table 2) suggest that FeRn expression/functional changes are not solely responsible for all changes in mAb disposition. Indeed, cardiovascular/hematologic changes during normal pregnancy also probably contribute. Specifically, the physiological PK simulation model of Garg and Balthasar (2007) suggests that normal plasma volume increases and blood pressure de-
creases in rat pregnancy (Dowell and Kauer, 1997) could lead to the IgG Vd and CIs 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). It is noteworthy that these hemodynamic changes in the rat model could mimic human changes (Dowell and Kauer, 1997), suggesting that mAb Vd and CIs changes may also occur during human pregnancy.

Although 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 probably reflect increase amounts of mAb-bound PCP, because tissue PCP levels directly reflect mAb levels (Laurenzana et al., 2003a,b; Pentel et al., 2006). Mother-to-fetus mAb transfer in this period is not surprising, because the rat blood-placental barrier maturation occurs between GD12 and GD15 (de Rijk et al., 2002). The decrease in fetal PCP levels from GD12 to 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) provide 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 seems to most efficiently antagonize PCP entry into the fetus after placental barrier maturation (Fig. 4). These functional data seem applicable to human pregnancy, because 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. Although 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 $t_{1/2}$ of mAb-bound drug (Lobo et al., 2004). Although fetal PCP data from GD9 confirm that mAb-bound drug can increase total drug levels, whereas 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). This could raise concerns about possible effects of higher mAb-associated fetal drug levels and an extended $t_{1/2}$ of mAb-bound drug (Lobo et al., 2004). Although fetal PCP data from GD9 confirm that mAb-bound drug can increase total drug levels, whereas 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 $t_{1/2}$ in mAb-dosed animals does not reflect an extended $t_{1/2}$ of the active drug, but instead of the inactive, mAb-bound drug. Even if a small amount of free drug is present because of the natural on-off process of mAb binding, previous work has shown that not all drugs 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 untreated (empty bar) and mAb6B5-treated (filled bars) groups. Tissue was collected at T -5 min, T 30 min, and T 8 h from mAb administration (*, p < 0.05 versus controls). C, bound (empty bars) and unbound, or “free,” (filled bars) 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).
bound PCP levels in mAb6B5-treated fetuses (Fig. 5C) demonstrate 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 antiaddiction medications (Fig. 4). It is noteworthy that the mAb6B5-mediated decreases in maternal brain PK concentrations reported here (95% confidence interval 39–53%; range 23–63%) were larger than the 20% reduction that protected male rats from adverse PCP effects (Laurenzana et al., 2003b). These maternal brain PK reductions levels carry potentially important implications for human treatment, because high brain levels consistently correlate with adverse effects (Kantak et al., 2000; Laurenzana et al., 2003b; Pentel et al., 2006). Although 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 such as 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 antidrug mAb or IgG seems 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 pregnant rat (or other 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 dosing and safety guidelines for mAb therapy in preclinical pregnancy treatment models (Mir et al., 2007; Kane and Acquah, 2009; Østensen and Förger, 2009). Our report also provides 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 maintain neuroprotective mAb steady-state levels throughout pregnancy, 2) both FeRn-mediated clearance and gestational hemodynamic changes seem 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 antidrug mAb in pregnancy seems to safely reduce maternal and fetal PCP exposure.

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Authorship Contributions

Participated in research design: Hubbard, Laurenzana, Gentry, and Owens.

Conducted experiments: Hubbard and Laurenzana.

Performed data analysis: Hubbard, Williams, and Owens.

Wrote or contributed to the writing of the manuscript: Hubbard and Owens.

Other: Hubbard and Owens secured funding for the research.

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**Address correspondence to:** S. Michael Owens, Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, 4301 W. Markham St., #611, Little Rock, AR 72205. E-mail: mowens@uams.edu