This is the background section of an NIH grant application.

A. BACKGROUND AND SIGNIFICANCE

Pregnancy affects the absorption, distribution, metabolism and elimination of pharmacologic substances. However, knowledge of how drug pharmacokinetics (PK) and pharmacodynamics (PD) are altered by pregnancy is limited,[1] and this hampers our ability to treat both women and fetuses. There are numerous challenges in implementing PK and PD study protocols in a population of pregnant women, including:

- The complexity of the maternal and fetal biological systems, comprised of a large number of interconnected components for which function in drug disposition, toxicity and safety is only partially understood
- The need for new biomarkers to rapidly and specifically assess issues related to safety, toxicity, and especially efficacy
- The ethical obstacles related to the design and conduct of clinical studies in this population
- The need to address the time dependent nature of PK and PD data, which requires development of pregnancy that account for nonlinearities with respect to system parameters, drug doses, and time.

There is an urgent need for a better understanding of the interplay of the factors influencing drug effects in the pregnant woman and the conceptus.

PK studies and drug trials in pregnant women have not been conducted routinely because of safety concerns. However, the safety of women and fetuses may be compromised by administration of off-label drugs in pregnancy without information about the efficacy and safety profile or the proper dosing during pregnancy.[1,2]

The lack of safety data is particularly concerning. The conceptus is most susceptible to teratogenic effects during the first 60 days of pregnancy.[3] In addition, adverse events that affect the fetus may not be detected during the course of a trial, but only long after exposure, necessitation data collection over a long period of time.[4]

It is well known that the majority of pregnant women in the western world are exposed to one or more drugs during pregnancy.[5,6,7] Mothers with chronic diseases may take one or more drugs throughout pregnancy, based on the judgment that the benefit of the drug to the mother outweighs the risk to the fetus; examples include treatment for seizure disorders and affective disorders.[5]

Analysis of PK and PD data gathered during pregnancy using compartmental and noncompartmental methods and population analyses may require development of novel model structures to account for the increased complexity of the underlying maternal-fetal biology. Though complex, these model-based methodological approaches hold substantial promise for facilitating experimental design[8] and data analysis in the presence of unfavorable signal-to-noise ratios.

Especially promising are population-based methods employing nonlinear mixed effects models.[9] These models can be used to quantify the influence of demographic, environmental, and genetic factors on the disposition and effect of drugs, and ultimately the dose-response relationship. Explicit modeling of the dose-exposure-response cascade has been identified as a potentially rewarding analytical tool for drug development,[9] impacting issues ranging from protocol development to individual patient dose adjustment. "Model-based drug development" was identified as an area of opportunity in the recent FDA white paper on the Critical Path to New Medical Products.[10]

Efficacy and effectiveness trial planning cannot be properly informed without knowledge of PK and PD, because the optimum doses and dosage intervals for pregnant women are unknown.[1] Fetal therapy via maternal drug administration is another field with only a small evidence base to date. A deeper understanding of factors affecting drug levels in the mother and fetus will greatly advance knowledge in this area.

Many investigators have addressed the complexity of the maternal and fetal systems affecting drug absorption and elimination. There are several independent placental systems for drug transport and metabolism, including plasma membrane carriers, biotransforming enzymes, and export pumps.[11] Placental enzyme systems and transporters include several CYP isoenzymes and other monooxygenases, uridine diphosphate-glucuronosyltransferases, sulfotransferases, multidrug resistance proteins, breast cancer resistance protein,

extraneuronal monoamine transporter, human equilibrative nucleoside transporters, dipeptide transporters, sodium-dependent multivitamin transporters, carnitine transporter and glutathione *S*-transferases.[2,11,12,13,14,15,16,17,18,19,20,21,22] P-glycoprotein (P-gp), expressed in brush border, is one of the best studied placental molecules, and functions to pump xenobiotics out of the fetal compartment.[23] During intrauterine life, the placenta can perform both Phase I and Phase II reactions, and can metabolize many xenobiotics.[15] The placenta's metabolic ability is less extensive than that of fetal liver and is largely limited to steroid metabolism.[24] Different classes and types of enzymes, hormone receptors, and transporters are located on the brush border than on the basal membrane, so that transfer of nutrients and waste products to and from the fetus can occur unidirectionally.[25] Fetal to maternal transfer of some drugs has been demonstrated.[5]

In addition to the placenta, fetal liver and extrahepatic tissues (adrenal, lung, kidney, spleen, thymus, brain, and cardiac tissues) contain several CYP forms. Many of these enzymes are characterized by adaptive enhancement, so that expression is governed by maternal health, environmental factors and gestational age.[5,24] The mechanisms governing constitutive and induced expression of CYPs in fetal tissues and placenta are still being characterized.[15]

Placental permeability to drugs is primarily via passive diffusion, and is influenced by many factors including ionization, maternal-fetal concentration gradient, surface area and thickness of the placenta, pH of maternal and fetal blood, extent of protein binding in maternal and fetal blood, lipid solubility, polarity, molecular weight, blood flow in the fetal and maternal compartments, and the formation of aggregates with similar molecules or other compounds.[5,14,15,26] Pharmacologic characteristics may act as modifiers of placental permeability. For example, although protein-bound drugs cannot cross the placenta by passive diffusion, binding is a transitory phenomenon, so that when the drug separates from the protein it can cross the placenta before binding to another protein[5]. Several penicillins, including ampicillin, diffuse across the placenta despite being strongly acidic.[27]

Other mechanisms of drug passage across the placenta are thought to be of lesser importance. Facilitated diffusion is a minor transfer mechanism.[5] Some drugs demonstrate a "depot phenomenon" in vitro when binding to placental tissue, but the extent to which this phenomenon occurs in vivo is not known.[5] Endocytosis has been considered a minor mechanism, and though this is now questioned in the case of new peptide and nucleic acid therapies, the internalization of viruses via endocytosis has been demonstrated.[5,28]

Placental transporters are not necessarily restricted to their physiological substrates. Xenobiotics with structures similar to physiological substrates may inhibit transport of these substrates by competition, interfering with fetal development.[5,11,29] In other cases, placental enzymes facilitate the production of metabolites that are more toxic to the fetus than the original compound. [5,11]

Placental metabolism and transport are affected by environmental factors, including drugs of abuse, nicotine, alcohol, air pollution and contaminated food. [5]

Therapy with multiple drugs may give rise to drug interactions in the placenta, and these phenomena may form the basis for optimization of pharmacotherapy. It is well known, for example, that verapamil administered for fetal tachyarrhythmia blocks placental efflux of digoxin by P-glycoprotein, thus enhancing digoxin delivery to the fetus.[5]

Maternal physiologic changes throughout pregnancy are numerous and have major effects on drug absorption, metabolism and elimination via changes in volume of distribution, transport proteins, clearance and half-life.[2] Glomerular filtration rate (GFR) increases throughout pregnancy, until about the 37th week.[2] Late pregnancy is characterized by changes in hepatic blood flow and protein binding.[2,30] Activity of some CYP isoenzymes is increased and others decreased during pregnancy, necessitating dosage changes.[2,31,32] The few studies that have been conducted on P-gp expression in human placental and fetal tissue suggest a change in its expression over the course of pregnancy.[33] Intestinal motility is decreased and gastric pH is increased during pregnancy, but whether these factors significantly alter drug absorption is unknown.[1,2] Cardiac output is elevated throughout pregnancy and plasma volume is increased.[2]

The placental structure itself changes dramatically during pregnancy, evidenced by thinning of the organ and accompanying increases in surface area and blood flow.[34] The integrity of the placenta or blood flow patterns and substance transfer may be altered in pathological situations such as hypertension and hydrops fetalis.[5]

The study of obstetric pharmacology presents several methodological challenges and problems. In vitro models include perfused cotyledons, villus explants and monolayer cultures, isolated trophoblast plasma membrane and isolated transporters and receptors, but these cannot account for all the variables present in vivo.[5] The cotyledon model has been used with success, but cannot account for protein binding effects and rates of efflux in vivo.[35]

Animal species demonstrate considerable heterogeneity in placentation and xenobiotic metabolism, so that lower animal models cannot be reliably used to predict drug behavior in humans.[15,34] In many experimental animals, the fetus is unable to metabolize drugs until late fetal or post-natal life, limiting the species that are useful for study.[36]

In vivo methods to characterize maternal-fetal drug transfer must administer the drug to both the fetal and maternal compartments and then measure drug concentration in both the maternal and fetal blood. Due to the high risks of human percutaneous umbilical blood sampling, this technique is limited to animal studies.[14] Plasma concentrations may not accurately predict intracellular concentrations.[37]

Studies performed in relatively small numbers of human subjects can form the basis for simulation of clinical trials that have not been conducted. This is usually accomplished through the formulation of a PK-PD model conditional on the available data. The parameter estimates and model structure thus developed can then be used to explore other dosing scenarios. Monte Carlo simulation allows for large numbers of hypothetical subjects, but still reflects the characteristics of the original population analyzed.[38] Though Monte Carlo methods are limited by the appropriateness of the PK-PD model, they are a powerful tool, in conjunction with PK-PD modeling, for rigorously comparing various drug administration strategies.

Human studies in this area are few, but there are several examples of successful work. Medical management of fetal SVT with digoxin or flecainide and digoxin has been accomplished.[39] Elliot's group[40] showed that glyburide did not cross the placental barrier in appreciable quantities, and conducted a randomized controlled trial on pregnancy outcomes comparing insulin to glyburide.[41] Koren's group has recently demonstrated glyburide efflux from the fetal to the maternal circulation by several transporters.[42] Kraemer et al. documented this in vitro in a placental perfusion model, and showed that verapamil does not modify glyburide transport, suggesting that P-gp is not the responsible transporter.[35] Andrew et al. characterized amoxicillin PK in pregnancy and three months postpartum and studied different dosage strategies, both with empiric data and model-based Monte Carlo simulations, demonstrating a potential need for modifications in dosing of amoxicillin for anthrax prevention.[38]

Studies should be prioritized by maternal/fetal risk and need, and PK studies should precede efficacy studies.[42] Assessments of the feasibility of using previously characterized substrates as markers for dosage adjustment are needed. Study designs should consider gestational age, since metabolic effects change throughout pregnancy.

H. REFERENCES

[1] Mattison D, Zajicek A. Gaps in knowledge in treating pregnant women. Gend Med 2006;3:169-82.

[2] Anderson DG. Pregnancy-induced changes in pharmacokinetics. Clin Pharmacokinet 2005;44:989-1008.

[3] Juchau, MR, Lee QP, Fantel AG. Xenobiotic biotransformation/bioactiviation in organogenesis-stage conceptal tissues: implications for embryotoxicity and teratogenesis. Drug Metab Rev 1992;24:195-238.

[4] Moretti M. Collection and analysis of drug safety data in pregnancy. Can J Clin Pharmacol 2007;14:e34-e36.

[5] Syme MR, Paxton JW, Keelan JA. Drug transfer and metabolism by the human placenta. Clin Pharmacokinet 2004;43:487-514.

[6] Glover DD, Amonkar M, Rybek BF, Tracy TS. Prescription, over-the-counter, and herbal medicine use in a rural, obstetric population. Am J Obstet Gynecol 2003;188:1039-45.

[7] Lee E, Maneno MK, Smith L, Weiss SR, Zuckerman IH, Wutoh AK, Xue Z. National patterns of medication use during pregnancy. Pharmacoepidemiol Drug Saf 2006:15:537-45.

[8] Hooker A, Vicini P. Simultaneous population optimal design for pharmacokinetic-pharmacodynamic experiments. AAPS J. 2005 Nov 1;7(4):E759-85.

[9] Sheiner LB, Beal SL. Evaluation of methods for estimating population pharmacokinetics parameters. I. Michaelis-Menten model: routine clinical pharmacokinetic data. J Pharmacokinet Biopharm. 1980 Dec;8(6):553-71.

[10] DHHS FDA. FDA's Critical Path Initiative: The Critical Path to New Medical Products. March 2004. http://www.fda.gov/oc/initiatives/criticalpath/ (accessed April 11, 2007)

[11] Marin, JJG, Briz O, Serrano MA. A review on the molecular mechanisms involved in the placental barrier for drugs. Curr Drug Deliv 2004 ;1 :275-89.

[12] Zwart R, Verhaag S, Buitelaar M et al. Impaired activity of the extraneuronal monoamine transporter system known as uptake-2 in Orct3/SIc22a3-deficient mice. Mol Cell Biol 2001;21:4188-96.

[13] Friedrich A, Prasad PD, Freyer et al. Molecular cloning and functional characterization of the OCTN2 transporter at the RBE4 cells, an in vitro model of the blood-brain barrier. Brain Res 2003;968:69-79.

[14] Unadkat, JD, Dahlin A, Vijay S. Placental drug transporters. Curr Drug Metab 2004;5:125-31.

[15] Hakkola J, Pelkonen O, Pasanen M, Raunio H. Xenobiotic-metabolizing cytochrome P450 enzymes in the human feto-placental unit : role in intrauterine toxicity. Crit Rev Toxicol 1998;28(1):35-72.

[16] Cordon-Cardo C, O'Brien JP, Boccia J, Casals D, Bertino JR, Melamed MR. Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. J Histochem Cytochem 1990;38:1277-87.

[17] Allikmets R, Schriml LM, Hutchinson A, Romano-Spika V, Dean M. A human placenta-specific ATP-binding cassette gene (ABCP) on chromosome 4q22 that is involved in multidrug resistance. Cancer Res 1998;58:5337-9.

[18] Ramamoorthy S, Leibach FH, Ganapathy V. Partial purification and characterization of the human placental serotonin transporter. Placenta 1993;14:449-61.

[19] Price NT, Jakson VN, Halestrap AP. Cloning and sequencing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past. Biochem J 1998;329:321-8.

[20] Pacifici GM, Rane A. Glutatnhione S-transferase in the human placenta at different stages in pregnancy. Drug Metab Dispos 1981;9:472-5.

[21] Friesema EC, Ganguly S, Abdalla A, Manning Fox JE, Halestrap AP, Viser TJ. Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. J Biol Chem 2003;278:41028-35.

[22] Awasthi YC, Sharma R, Singhal SS. Human glutathione S-transferases. Int J Biochem 1994;26:295-308.

[23] Fricker G, Miller DS. Relevance of multidrug resistance proteins for intestinal drug absorption in vitro and in vivo. Pharmacol Toxicol 2002;90:5-13.

[24] Pasanen M. The expression and regulation of drug metabolism in human placenta. Adv Drug Deliv Rev 1999;38:81-97. Adv Drug Deliv Rev 1999;38:81-97.

[25] Sideri M, de Virgiliis G, Rainoldi R, Remotti G. The ultrastructural basis of the nutritional transfer : evidence of different patterns in the plasma membrances of the multilayered placental barrier. Trophoblast Res 1983;1:15-26.

[26] Slikker W, Miller RK. Developmental Toxicology. Raven Press, Ltd.,, New York, pp. 245-283.

[27] Pacifici GM, Rane A. Glutatnhione S-transferase in the human placenta at different stages in pregnancy. Drug Metab Dispos 1981;9:472-5.

[28] Phillips DM, Tan X. AIDS Res Hum Retroviruses 1992;8:1683-91.

[29] Ganapathy V, Prasad PD, Ganapathy ME, Leibach FH. Placental transporters relevant to drug distribution across the maternal-fetal interface. J Pharmacol Exp Ther 2000;294:413-20.

[30] Hill MD, Abramson FP. The significance of plasma protein binding on the fetal/maternal distribution of drugs at steady-state. Clin Pharmacokinet 1988;14:156-70.

[31] Dempsey D, Jacob P, Benowitz NZ. Accelerated metabolism of nicotine and cotinine in pregnant smokers J PET 2002;301:594-8.

[32] Heikkinen T, Ekblad U, Palo P, Laine K. Pharmacokinetics of fluoxetine and norfluoxetine in pregnancy and lactation. Clin Pharmacol Ther 2003;73:330-7.

[33] MacFarland A, Abramovich DR, Ewen SW et al. Stage-specific distribution of P-glycoprotein in first trimester and full-term human placenta. Histochem J 1994;26:417-23.

[34] van der Aa EM, Peereboom-Stegeman JHJC, Noordhoek J, Gribnau FW, Russel FG. Mechanisms of drug transfer across the placenta. Pharm World Sci 1998;20:139-48.

[35] Kraemer J, Klein J, Lubetsky A et al. Perfusion studies of glyburide transfer across the human placenta: implications for fetal safety. Am J Obstet Gyencol 2006;195:270-4.

[36] Pelkonen O, Breimer DD. Handbook of Experimental Pharmacology. Karger, Basel, 1994, page 289.

[37] Craig WA. Does the dose matter? Clin Infect Dis 2001;33:S233-237.

[38] Andrew MA, Easterling TR, Carr DB, Shen D, Buchanan ML, Rutherford T, Bennett R, Vicini P, Hebert MF. Amoxicillin pharmacokinetics in pregnant women: modeling and simulations of dosage strategies. Clin Pharmacol Ther 2007;81:547-56.

[39] Dimas VV, Taylor MD, Cynnyngham CB et al. Transplacental pharmacokinetics of flecainide in the gravid baboon and fetus. Pediatr Cardiol 2005;26:815-20.

[40] Elliott BD, Schenker S, Langer O, Johnson R, Prihoda T. Comparative placental transport of oral hypoglycemic agents in humans. Am J Obstet Gynecol 1994;171:653-60.

[41] Langer O, Conway DL, Berkus MD, Xenakis EM, Gonzales O. A comparison of glyburide and insulin in women with gestational diabetes mellitus. N Engl J Med 2000 ;343 :1134-8.

[42] Koren G, MacLeod S, Davis D. Drugs in pregnancy: acknowledging challenges—finding solutions. Can J Clin Pharmacol 2007;14:e2-e4.