Ontogeny of Rat CYP2E1 and CYP1A2: A Characterization and a Pharmacokinetic Model

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ABSTRACT

Infantile exposure to xenobiotics, e.g. from breastfeeding, poses a serious toxicity risk. Since the toxicokinetic mechanisms that principally determine exposure outcomes undergo a significant developmental maturation, infants may respond to exposures in a different way than adults. Hence, suitable model systems are required to provide risk relevant information in pediatric populations. This dissertation's primary goal was to provide a critical evaluation of two such model systems; first, a pharmacokinetic model that may predict an infant's capacity to eliminate toxicants by cytochrome P-450 (CYP) mechanisms and second, the developing rat as a model of human CYP2E1 and CYP1A2 ontogeny.

The first objective was to evaluate underlying assumptions of a pharmacokinetic model that describes the ontogeny of hepatic CYP activity using the rat. The study recognized some discrepancies with the stated assumptions. The impact of these discrepancies on the potential applicability of the model is discussed. As proof-of-concept, the observed data were fit to a model describing rat CYP2E1 and CYP1A2 ontogeny. A reasonable correlation ($r = 0.75$) was observed between observed and predicted oral clearance values of a CYP2E1 substrate indicating the potential applicability of such a model in risk assessment.

The second objective was to conduct an extensive characterization of rat hepatic CYP2E1 and CYP1A2 ontogeny at mRNA, protein, activity and intrahepatic expression levels. The results were compared to available human data to determine the appropriateness of the rat for assessment of toxicokinetic mechanisms underlying age-dependent differences in susceptibility to toxicity. Similarities in age-dependent changes in mRNA, activity and zonal hepatic expression patterns were noted between the rat and human prior to weaning. Unlike human data, rats show good correlation between changes in CYP2E1 and CYP1A2 activity and transcript levels, but not with the immunoquantifiable protein. Recognizing such similarities and differences between rats and human regarding onset, rate and pattern of CYP ontogeny will

improve the accuracy of rat-to-human extrapolation of developmental toxicokinetic data.

Overall, the dissertation research provides mounting and supportive evidence for the use of such model systems in providing risk-relevant information in pediatric populations and to identify toxicokinetic mechanisms underlying age-dependent differences in susceptibility to toxicity.

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±	plus or minus
°C	degree Celsius
AAG	$alpha_1$-acid glycoprotein
ADME	Absorption, distribution, metabolism and elimination
AhR	aryl hydrocarbon receptor
AIC	akaike information criterion
AUC_∞	area under the serum concentration-time curve
ARNT	aryl hydrocarbon receptor nuclear translocator
BW	body weight
BrW	brain weight
CAR	constitutive androstane receptor
CDNB	1-chloro-2,4 dinitrochlorobenzene
CI	confidence interval
Cl/F	oral clearance
Cl_H	hepatic clearance
Cl_{int}	intrinsic clearance
$Cl_{S,Inf}$	infant systemic clearance
$Cl_{S,Mat}$	maternal systemic clearance
C_{max}	maximum serum concentration
Css_{Inf}	infant steady state serum concentration
$Css_{,Mat}$	maternal steady state serum concentration
CYP	cytochrome P450
CZX	chlorzoxazone
DAB	3,3' diaminobenzidine hydrochloride
DME	drug metabolizing enzymes
$Dose_{Inf}$	drug dose given to the infant
EDTA	ethylenediamine tetra acetate
$EI_{(Conc)}$	exposure index related to maternal serum concentration
$EI_{(Dose)}$	exposure index related to maternal dose
E_H	hepatic extraction ratio
EPA	US environment protection agency
ER	endoplasmic reticulum
EROD	ethoxyresorufin dealkylase
F	bioavailability
FDA	US Food and Drug Administration
FFA	free fatty acids
4-NC	ρ-nitrocatechol
f_u	fraction of the drug unbound in plasma
GH	growth hormone
GIT	gastrointestinal tract
GSH	glutathione

GST	glutathione-S-transferases
HAAs	heterocyclic aromatic amine
HC	Health Canada
HNF	hepatocyte nuclear factor
HPLC	High Pressure Liquid Chromatography
HSF	hepatic scaling factor
IHC	immunohistochemistry
ISF	infant scaling factor
ISF_D	a fitted parameter for the model equation
IV	intravenous
k	first-order rate constant
K_M	Michaelis-Menten constant
λ_z	terminal elimination rate constant
LOD	limit of detection
LOQ	limit of quantitation
LW	liver weight
MC	3-methylcholanthrene
MeIQx	2-amino-3,8-dimethylimidazole[4.5]quinoxaline
mg	milligram(s)
min	minute(s)
µL	microliter(s)
mL	milliliter(s)
µm	micrometer(s)
mm	millimeter(s)
mM	millimole(s) per liter
MLP	maximum lifespan potential
MP	microsomal protein
MR	methoxyresorufin
MROD	methoxyresorufin-O-dealkylase
(M/S)	milk to serum ratio
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
NAPQI	N-acetyl-p-benzoquinoneimine
NAT	N-acetyl transferases
NF	nuclear factor
nM	nanomole(s) per liter
NSAIDs	non-steroidal anti-inflammatory drugs
OSF	ontogeny scaling factor
PAGE	polyacrylamide gel electrophoresis
PAHs	polycyclic aromatic hydrocarbons
PAPS	3'-phosphoadenosine-5'-sulphophosphate
PBPK	physiologically-based pharmacokinetic
PBS	phosphate buffered saline
PBSt	phosphate buffered saline with 0.05 % tween 20
PD	postnatal day
pH	negative logarithm of hydrogen ions to the base 10
PK	pharmacokinetics

PNP	ρ-nitrophenol
POD	phenacetin-O-dealkylase
PP	periportal
psi	pounds per square inch
PV	perivenous
PVDF	polyvinylidene difluoride
PXR	pregnane-X-receptor
QC	quality control
Q_H	hepatic blood flow
r^2	coefficient of determination
RHSF	relative hepatic scaling factor
RSD	relative standard deviation
RT-PCR	reverse transcription polymerase chain reaction
SD	standard deviation
SDS	sodium dodecyl sulfate
SULT	sulfotransferases
τ	infant nursing interval
$t_{1/2}$	Elimination half life
TCDD	2,3,7,8 tetrachlorodibenzo-p-dioxin
t_{max}	time to reach maximum serum concentration
UDP	uridine 5'diphosphate
UGTs	uridine 5'diphosphate glucuronosyl-transferases
UMB	umbelliferone
UV	ultraviolet
V_d	volume of distribution
$V_{d,ss}$	apparent volume of distribution at steady-state
V_{max}	maximal enzyme activity
V_{Milk}	volume of milk ingested by infant

1. LITERATURE REVIEW

I. INTRODUCTION

Epidemiological research provides convincing evidence that breastfeeding is advantageous to the breast-fed infant, lactating mother and society. However, the ability of most drugs and chemicals to transfer from maternal plasma to breast milk puts the suckling infant under an uncertain exposure risk to harmful xenobiotics and raises concerns about breastfeeding safety. When a nursing mother is exposed to a xenobiotic(s), either from the environment or through drug therapy, a decision to discontinue the exposure (e.g. stop medication) or discontinue breast feeding is largely based on data regarding the ability of the drug to appear in breast milk, the amount transferred to the suckling infant and, more importantly, the ability of the suckling infant to handle such an exposure. Despite the growing information about the ability of chemicals to transfer into breast milk and the numerous methods developed to estimate the amount exposed to the suckling infant, very little is known regarding the ability of the infant to deal with the exposed dose. Following an external exposure dose, pharmacokinetic processes determine the concentration of a xenobiotic in the biophase. In many instances it is the capacity of the detoxification mechanisms to eliminate a xenobiotic that poses as the major underlying cause for a toxic outcome following an exposure.

Detoxification systems undergo significant maturation during postnatal development. In human paediatric populations, such age-related differences in elimination mechanisms are generally poorly understood. However, for a given dose, the rate and pattern of the ontogeny of elimination processes will alter the toxicological significance of a xenobiotic in an age-dependent manner and such developmental maturation will confound the extrapolation of toxicity data obtained in adults to the

developing infant. Although the recent establishment of the Children's PK Database (available at http://www2.clarku.edu/faculty/dhattis), a database presently under construction to identify differences in pharmacokinetics during development, facilitates extrapolation of risks between adults and infants, this database still falls short of providing sufficient information for the adequate extrapolation of toxicity data, particularly for environmental toxicants.

Interspecies extrapolation of toxicity studies performed with the developing animal is one potential approach proposed to elucidate the impact of the maturation of toxicokinetic processes on age-dependent differences in susceptibility to toxicity and to allow inclusion of risk-relevant information on the developing animal into risk assessment models. An animal model that can, at the very least, identify an underlying toxicokinetic mechanism for age-dependent differences in susceptibility to toxicity has great value in risk assessment. However, because of the interrelationship between genetic and environmental factors that influence the pattern and rate of xenobiotic elimination, a perfect animal model is unlikely to be found. Relevant differences will always exist between human and animal models and these differences will pose serious challenges for qualitative and quantitative predictions of human toxicological outcomes. Translating animal data to humans requires an understanding of how and where these systems are similar and different to evaluate their impact on extrapolation to human exposure risks.

This dissertation takes a systems level approach (i.e. evaluation of mRNA, protein and function) in the evaluation of two detoxification mechanisms, Cytochrome P450 (CYP) 2E1 and CYP1A2. This evaluation will provide critical descriptive information necessary to known information on human CYP ontogeny and to draw comparisons that determine the appropriateness of the developmental animal model system for assessment of toxicokinetic mechanisms underlying age-dependent differences in susceptibility to toxicity. Furthermore, this dissertation evaluates critical assumptions of a pharmacokinetic model that describes the ontogeny of CYP enzymes for its application as a risk assessment tool when infants are exposed to xenobiotics present in breast milk. Literature to date indicates that CYP enzymes in humans are regulated in a similar manner to rodents[1]. Therefore rodents are frequently used to provide initial information

about xenobiotic elimination in humans. Accordingly, a rat model system was used to evaluate the underlying assumptions of a pharmacokinetic ontogeny model of CYP enzyme-mediated clearance. Furthermore, rat and human CYP2E1 and CYP1A2 enzymes exhibit high homology and similar substrate profiles[2-4]. This could allow use of the rat as an appropriate model to study the developmental expression and function of human CYP2E1 and CYP1A2.

For the purpose of this literature review, the term infant encompasses ages from birth to 2 years and children from 2 years to 12 years of age in humans. However, the term neonate includes birth to postnatal day 21, weanling indicates postnatal days 21 to 28/35 and juvenile encompasses age from postnatal days 28/35 to 49/70 days for rat.

II. BACKGROUND

1. Breastfeeding and Infant Exposure to Chemicals

1.1 Breastfeeding and its Benefits

Extensive epidemiological research has identified diverse and compelling advantages of breastfeeding for infants, mothers, families, and societies and the use of human milk for infant feeding[5]. These advantages include health, social, environmental, and economic benefits[5]. Thus, current guidelines set out by international (i.e. World Health Organization and United Nations Children's Fund) and national (i.e. Canadian Pediatric Society, Health Canada and Dietitians of Canada) organizations recommended that infants be exclusively breastfed for at least the first six months of life, and continue breastfeeding with complementary foods for up to two years of age and beyond[6-8]. Surveys in Canada and United States report that approximately 70% of women initiate breastfeeding and 30% continue to breastfeed their infants to 6 months of age[5].

The unique components of human milk make it superior for infant feeding, and differs markedly from all substitute feeding preparations[9]. The nutrients, enzymes, growth factors, hormones and immunological and anti-inflammatory properties of

human milk contribute to reductions in the incidence and severity of acute infectious diseases such as sepsis/meningitis[10], otitis media[11], respiratory tract infections[12], gastroenteritis[11] and necrotizing enterocolitis[13]. As well, studies correlate breastfeeding with reduced incidences of sudden infant death syndrome[14,15], Crohn's disease, insulin-dependent diabetes, lymphoma and allergic diseases[13,15,16] and reduced prevalence of childhood obesity[17,18]. Furthermore, breastfeeding may enhance performance on tests of cognitive developments and achievement of better academic scores[19,20]. Such health benefits may increase with duration and exclusivity of breastfeeding[21].

Breastfeeding and lactation have many important benefits for nursing women. These benefits include decreased postpartum bleeding and more rapid uterine involution[22], reduced risk of epithelial ovarian cancer[23] and breast cancer[24,25], reduced risk of hip fracture in old age[26,27] and faster return to the pre-pregnancy weight [28]. The act of breastfeeding delays resumption of ovulation and increases child spacing[29]. Finally, breastfeeding is associated with increased maternal-infant bonding and maternal sense of worth[5].

Breastfeeding has important social, economic and environmental benefits. These benefits include reduced health care costs due to reduced risk of infant illness and duration of hospitalization[11,13]. Also breastfeeding is associated with increased family saving due to the greater cost of formula feeding compared to breastfeeding cost[11] in addition to lower parental absence from work, and reduced environmental load for disposing formula cans and bottles[5]. Some countries, including Canada, have recognized the potential economical savings from breastfeeding and provide a subsidy to low income mothers for each month of breastfeeding[11].

1.2 Infant Exposure to Drugs and Chemicals in Breast Milk

Almost all maternally administered drugs and environmental chemicals appear in breast milk and find their way into the breast-fed infant[30-33]. Consequently, breast milk constitutes a major exposure route to chemicals for nursing infants. Governmental and regulatory agencies have become gravely concerned about the presence of chemicals in breast milk, particularly in light of recent surveys that suggest approximately 90% of

women take at least one medication, more than 20% take at least two or more medications in the first week after delivery and more than 5% of nursing mothers receive long-term drug therapy[30,34] (Table 1.1).

Table 1.1- Example of maternally-administered medications that my have possible effects on the nursing infant. Adapted from references (37-39)

Drug	Reported or possible Effects on the Nursing Infant
1. Cytotoxic Drugs	
Cyclosporine	Possible immune suppression, carcinogenesis
Doxorubicin	Possible immune suppression, carcinogenesis
Methotrexatae	Possible immune suppression, carcinogenesis
2. Drugs of Abuse	
Amphetamine	Irritability, poor sleeping pattern
Cocaine	Intoxication, irritability, seizures, diarrhea
Heroin	Tremors, restlessness, poor feeding
3. Cardiovascular Drugs	
Acebutolol	Hypotension, bradycardia, tachypnea
Atenolol	Cyanosis, bradycardia
Amiodarone	Possible hypothyroidism
4. Antipsychotics	
Chlorpromazine	Drowsiness, decline in developmental scores, lethargy
Haloperidol	decline in developmental scores
5. Antibiotics	
Amoxicillin	None
Nalidixic acid	Hemolysis in infants with glucose-6-phosphate-dehydrogenase (G-6-PD)deficicncy.
Nitrofurantoin	Hemolysis in infants with (G-6-PD)deficicncy.
Tetracycline	Negligible absorption by infant
6. Others	
Phenobarbital	Sedation, infantile spasms
Sulfasalazine	Bloody diarrhea
Caffeine	Irritability, poor sleeping pattern, slower elimination