1521-009X/44/7/916–923$25.00
Drug Metabolism and Disposition

http://dx.doi.org/10.1124/dmd.116.071159
Drug Metab Dispos 44:916–923, July 2016

DRUG METABOLISM & DISPOSITION

Special Section on Pediatric Drug Disposition and Pharmacokinetics—Commentary

Challenges and Opportunities for Increasing the Knowledge Base Related to Drug Biotransformation and Pharmacokinetics during Growth and Development

J. Steven Leeder and Bernd Meibohm

Division of Clinical Pharmacology, Toxicology and Therapeutic Innovation, Department of Pediatrics, Children's Mercy Hospitals and Clinics, University of Missouri-Kansas City, Kansas City, Missouri (J.S.L.); and Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Sciences Center, Memphis, Tennessee (B.M.)

Received April 21, 2016; accepted May 11, 2016

ABSTRACT

It is generally acknowledged that there is a need and role for informative pharmacokinetic models to improve predictions and simulation as well as individualization of drug therapy in pediatric populations of different ages and developmental stages. This special issue contains more than 20 papers responding to the challenge of providing new information on scaling factors, ontogeny functions for drug metabolizing enzymes and transporters, the mechanisms underlying the observed developmental trajectories for these gene products, age-dependent changes in physiologic processes affecting drug disposition in children, as well as in vitro and in vivo studies describing the relative contribution of ontogeny and genetic factors as sources of variability in drug disposition in children. Considered together, these contributions serve to illustrate some of the current limitations regarding sample availability, number, and quality, but also provide a framework that allows for the potential value of the results of a given study to be interpreted within the context of these limitations. Among the challenges for the future are improving our understanding of the mechanisms regulating age-dependent changes in factors influencing drug disposition and response, thereby facilitating generalization to systems lacking detailed data, better integrating age-dependent changes in pharmacokinetics with age-dependent changes in pharmacodynamics, and allowing better predictability and individualization of drug disposition and response across the pediatric age spectrum.

Introduction

Although senescence contributes to age-related changes in drug clearance in adults, the magnitude of age-related changes associated with growth and development, or ontogeny, has considerably more potential to impact drug disposition in newborns, infants, toddlers, children, and adolescents. Unfortunately, this reality may not be immediately evident to investigators whose experience is limited to working with adult populations. Knowledge of the myriad factors contributing to variability in the dose-exposure knowledge base is much more extensive for adults compared with pediatric age groups, and therefore it makes sense to use existing adult data derived wherever possible to characterize drug disposition in children.

Compared with adult populations, pharmacokinetic (PK) assessments in children are often limited by logistic, physiologic, and sometimes even ethical constraints, particularly in the very young age groups. Thus, PK data in young pediatric patients are often sparse with regard to the number of samples, limited with regard to the sampling interval and specific sampling times, and small with regard to the sample volume. As a consequence, approaches using numerical modeling and simulation, i.e., pharmacometrics-based in silico tools, have often been promoted to support the characterization of the pharmacokinetics of novel and established therapeutic agents in children (Läer et al., 2009). These tools facilitate the development of dosing recommendations in different pediatric age groups and potentially even the individualization of drug dosing according to the needs of the individual patient (Vinks et al., 2015). In drug development and regulatory sciences, modeling and simulation are widely used as a rational approach for characterizing the dose-exposure and the exposure-response relationships in adults, and also for extrapolating them from adults to children. Mehrotra et al. (2016) have provided a compilation of an array of case studies for model-based analyses performed by the U.S. Food and Drug Administration's Office of Clinical Pharmacology in support of the approval of a variety of small molecule drugs and therapeutic proteins in pediatric patients (http://www.fda.gov/downloads/AdvisoryCommittees/

dx.doi.org/10.1124/dmd.116.071159.

ABBREVIATIONS: BPA, bisphenol A; DME, drug metabolizing enzyme; E_H, hepatic extraction; FMO, flavin monooxygenase; HPGL, hepatocytes per gram of liver; MPPGL, microsomal protein per gram of liver; NICHD, National Institute of Child Health and Human Development; NIH, National Institutes of Health; NQO1, NADPH-dependent quinone reductase; P450, cytochrome P450; PBPK, physiologically based pharmacokinetic; PK, pharmacokinetic; PMA, postmenstrual age; PNA, postnatal age; PXR, pregnane X receptor.

CommitteesMeetingMaterials/Drugs/AdvisoryCommitteeforPharmaceuticalScienceandClinicalPharmacology/UCM306989.pdf&sa=U&ei=HgFpU9fpLZD2oATv2oKYAg&ved=0CB0QFjAA&sig2=8E8ceyqCN-Q0AUEvix5KsQQ&usg=AFQjCNGjF0hPA5KwWEKd2f3uZNb_DCc-GQ).

For esomeprazole used in the treatment of gastroesophageal reflux disease, for example, population PK modeling was used to explore pediatric dosing regimens that result in similar steady-state esomeprazole exposure as observed in adults since the exposure-response relationship had been described as being comparable between the two groups. Similarly, for the anti-seizure compound vigabatrin, assessments of the dose-response relationship indicated similarity between adults and pediatric patients. Based on this observation, model-based exposure matching was considered sufficient and the need for a dedicated efficacy trial in children was alleviated. For the anti-TNF-α monoclonal antibody adalimumab, pharmacometric analyses in combination with observed efficacy and safety data provided evidence for the selection of a high-dose group and weight band–specific dosing. For the human immunodeficiency virus protease inhibitor darunavir, PK simulation studies allowed establishing a once-daily dosing regimen within the safety limits relative to peak and systemic exposure established by clinical twice-daily dosing data in 3- to 12-year-old human immunodeficiency virus–infected patients. Altogether, these examples illustrate wide applicability and substantial impact on regulatory decisions of modeling and simulation approaches for medications used in pediatric indications.

Modeling and Simulation Approaches

A common strategy to facilitate extrapolation to children is to apply a scaling factor to adult data; the allometric power model, as one example, calculates clearance for an individual child as the product of adult clearance and a scaling factor derived from expressing the weight of the child relative to a standard adult weight, usually 70 kg, expressed to the power 3/4 [i.e., $CL_{adult} \times (W_i/70\ kg)^{3/4}$] (Anderson and Holford, 2008). This approach performs fairly well once clearance pathways are fully mature (Edginton and Willmann, 2006), leading to the conclusion that "children are small adults, neonates are immature children" (Anderson and Holford, 2013). An alternative to allometric scaling, or complementary approach, is the use of models that incorporate functions describing the maturation of drug elimination pathways. One such approach that has gained popularity recently is physiologically based PK (PBPK) modeling used to predict drug disposition in pediatric populations (Barrett et al., 2012). Physiologically based models are systems of differential equations including a large number of parameters that can be classified as drug and system related; for pediatrics, the latter includes developmental changes in tissue volumes and composition, organ blood flow, gastric acidity, intestinal transit time, protein binding concentration, among others. Furthermore, developmental trajectories for several drug metabolizing enzymes (DMEs) have been characterized in vitro and in vivo, and ontogeny functions have been incorporated within commercially available PBPK platforms, such as Simcyp, GastroPlus, and PKSim (Johnson and Rostami-Hodjegan, 2011; Abduljalil et al., 2014; Samant et al., 2015).

Rioux and Waters (2016) reviewed the experience with PBPK modeling as a quantitative, systems pharmacology-based framework to support drug development in the specialized area of pediatric oncology. Their review summarizes the current experience with PBPK modeling in cancer drug development for children, and highlights the general opportunities, challenges, and limitations of the technology. With two more in-depth case studies on pinometostat, a DOT1L inhibitor for the treatment of mixed lineage leukemia, and tazemetostat, an EZH2 inhibitor for the treatment of multiple types of solid tumors, the authors exemplify model building workflow, model optimization, and sensitivity analyses routinely used in PBPK modeling.

Uncertainty and Variability in the Development of Pediatric PK Models

Theoretically, pediatric models can be built with existing in vivo data when available, the so-called top-down approach, or they can be generated from the bottom-up in which knowledge and structure are used to generate data; when applied to drug clearance, bottom-up approaches use a variety of scaling factors to extrapolate in vitro drug biotransformation data to in vivo clearance estimates (Rostami-Hodjegan and Tucker, 2007). A middle-out approach in which information from in vitro or in silico experiments is integrated with in vivo data has the potential to produce more mechanistically sound models that may be more relevant clinically (Tsamandouras et al., 2015). The process of in vitro-in vivo extrapolation for development of bottom-up PBPK models of drug clearance in children involves three scaling factors: one that addresses developmental changes in protein abundance [e.g., drug metabolizing cytochrome P450 (P540) enzymes and transporters]; one that scales in vitro intrinsic clearance of unbound drug to hepatic clearance; and one that addresses the changes in liver mass relative to total body mass that accompanies growth and development. Related to these scaling factors, it is important that distinctions be made between uncertainty and variability when interpreting the output of any pediatric model.

Uncertainty can be derived from the potential imprecision related to parameter estimation and implicit assumptions that may be based on limited data, such as the number of data points used to provide estimates of the various scaling factors, or whether these scaling factors themselves are subject to change with growth and development. For example, when cultured hepatocytes are used to predict clearance, the number of hepatocytes per gram of liver (HPGL), or hepatocullarity, is one such scaling factor. Using the NADPH-P450 reductase method to estimate HPGL, Fattah et al. (2016) report that HPGL is significantly (approximately 2-fold) higher in rat pups compared with adults, and progressively declines once the weaning period ends. Their data also provide insight into the ontogeny of NADPH-P450 reductase activity, which increases 2- to 2.5-fold over this same time period when expressed relative to 10^6 cells. These new HPGL and NADPH-P450 reductase activity data will be valuable for improving modeling and simulation of hepatic drug clearance in Wistar rats. However, more work in this area is required. Another important scaling factor is an estimate of microsomal protein per gram of liver (MPPGL), which is required for scaling intrinsic clearance of unbound drug to hepatic clearance. A recent investigation involving 128 liver samples from adults reported that MPPGL values were not normally distributed and varied 19-fold from 6.7 to 128.0 mg/g liver (Zhang et al., 2015). Unfortunately, MPPGL data derived from pediatric livers is extremely limited. Commonly cited studies of pediatric MPPGL are based on data from one (Barter et al., 2007) and four additional (Barter et al., 2008) pediatric liver samples less than 18 years of age, and available data regarding the developmental trajectory of MPPGL include only five data points between birth and adulthood. A similar situation exists for the cytosolic protein per gram of liver, where the adult cytosolic protein value of 80.7 mg/g of liver (Cubitt et al., 2011) is used for pediatric extrapolations since no corresponding pediatric scalar for the cytosolic protein per gram of liver is present in the literature (Rougée et al., 2016).

Variability can be derived from ontogeny, genetic variation, and environmental factors that are fundamental properties of the system (Tsamandouras et al., 2015). In fact, although PBPK modeling increasingly is being applied to improve prediction of drug clearance in children for pediatric drug development and regulatory purposes (Vinks

et al., 2015), skepticism has been expressed, largely due to the paucity of data regarding the developmental trajectories of transporters in particular as well as limited prospective evaluation and validation of models with clinical data. Even where developmental trajectories are available, values are usually limited to average data for a specific population or relative to a specific independent variable such as age. However, between-patient variability in these trajectories and their multifactorial interdependencies are rarely if ever known and further complicate the predictive value of PBPK models, particularly at the level of the individual patient.

Developmental Trajectories of DMEs and Transporters

Several papers in this special issue add to the knowledge base regarding the developmental trajectories of DMEs and transporters. Pearce et al. (2016) describe the role of ontogeny and genetic variation as determinants of variability of CYP2B6 activity in 24 prenatal, 141 pediatric, and 36 adult liver samples. CYP2B6 expression and activity was highly variable (mRNA, ~40,000-fold; protein, ~300-fold; activity, ~600-fold), and was significantly associated with age, with adult activity as measured by bupropion activity achieved by 1 year of age. Additional factors contributing to variability in activity remain to be identified since no significant effect of *CYP2B6* genotype, gender, and ethnicity was observed. Hines et al. (2016) investigated the developmental trajectory of cytosolic and microsomal CES1 and CES2 immunoreactive protein in 165 pediatric liver samples ranging in age from 1 day to 18 years of age. Using regression tree analysis to identify potential age thresholds, expression of both proteins was lower in samples with postnatal ages (PNAs) <3 weeks of age in both microsomes and cytosol relative to older ages; pronounced differences in protein expression among ethnic groups were also observed. Overall, the data imply that CES1- and CES2-dependent clearance of drugs and toxicants may be compromised in infants less than 3 weeks of age until expression fully matures. Similarly, Rougée et al. (2016) present data describing the ontogeny of NADPH-dependent quinone reductase (NQO1) in the cytosolic fraction of 117 liver samples obtained from pediatric (defined as 20 years of age or under, $n = 29$), adult ($n = 71$), and geriatric ($n = 71$) donors. A modest decrease in NQO1 immunoreactive protein over the entire age range was observed, but activity measured using 2,6-dichloroindophenol as the substrate was invariant with age. No gender-related differences in activity were observed, but activity was reported to be higher in Caucasians compared with Asians. Of interest, NQO1 activity was higher in overweight children [defined as body mass index (>85th percentile for age and gender)] compared with ideal weight children, whereas activity was lower in obese adults (body mass index >30 kg/m^2) compared with ideal weight adults. In contrast to protein quantitation using immunoblot analysis, Chen et al. (2016) developed a liquid chromatography–tandem mass spectrometry and multiple reaction monitoring–based targeted proteomic method to characterize flavin monoxygenasse (FMO)1, FMO3, and FMO5 in seven prenatal (14–20 weeks gestation), 16 pediatric (aged 5 months to 10 years), and 10 adult liver samples. The data presented confirm the established developmental trajectories for FMO1 and FMO3 determined by immunoblot analysis, but the limited number of pediatric samples precludes more definitive characterization of the FMO ontogeny. However, application of the proteomic method provided evidence that the ratio of holoprotein to total protein was greater in FMO5 baculosomes compared with FMO3, and may have value in clarifying the relationship between protein expression and catalytic activity in different in vitro drug biotransformation systems (Chen et al., 2016).

As current immunoblot- and proteomic-based approaches for quantifying protein content do not distinguish between functional, catalytically active enzyme and inactive (apoprotein) forms, Sadler et al. (2016) applied a novel activity-based protein profiling approach to characterize the developmental trajectory of several P540 enzymes simultaneously in hepatic microsomes prepared from prenatal ($n = 30$), neonate/infant (<1 year), juvenile (ages 1–18 years; $n = 10$), and adult (> 18 years; $n = 9$) livers. The approach relies upon the catalytic activity of functionally active P540 enzymes to bioactivate modified mechanism-based inhibitors to covalently modify the bioactivating P540 enzymes, which are subsequently identified by liquid chromatography mass spectrometry–based proteomics or fluorescent gel imaging techniques. Using this approach, the expected high prenatal expression of CYP3A7 and decline after birth was replicated, and CYP4A11, 4F12, 19A1, and 51A1 were also found to share this same developmental pattern. CYP4F2, 4F3, 7B1, 8B1, and 20A1 demonstrated similar levels of activity in prenatal and postnatal liver, whereas activity of CYP2A6, 2B6, 2C8, 2C19, 2J2, 3A5, 4F11, 4V2, and 27A1 is low prenatally but increases after birth. The developmental profile of CYP2B6 determined by activity-based protein profiling agrees with the results of Pearce et al. (2016) described previously. It is anticipated that proteomic-based analyses of protein abundance will lead to more accurate ontogeny functions for bottom-up PBPK modeling compared with immunoblot data; however, application of activity-based protein profiling to a larger number of samples will be required to determine if ontogeny functions derived from active protein content will prove to be superior to proteomic data.

One of the more pronounced knowledge deficits in pediatric drug disposition and pharmacokinetics is the ontogeny of drug transporters (Brouwer et al., 2015). In this special issue, Elmorsi et al. (2016) briefly summarize the current state of knowledge, including a list of drugs commonly used in pediatrics that are substrates for various transporters. Simulations are presented to illustrate how the relative contribution of competing transporters (i.e., the concept of fraction transported) may be influenced by relative abundance and ontogeny during growth and development, and to emphasize the importance of addressing this deficit to improve modeling and simulation in children.

In this special issue, Thomson et al. (2016) describe the developmental trajectory of OATP1B1 and OATP1B3, products of the *SLCO1B1* and *SLCO1B3* genes, respectively. In a set of pediatric livers up to 12 years of age ($n = 80$) consisting of samples from 32 living donors and 48 post-mortem samples, immunoreactive OATP1B1 expression was invariant with age, although a tendency toward higher expression was observed in the older age group (6–12 years of age); this higher expression did not achieve statistical significance, likely due to the wide interindividual variability observed within this age group. In contrast, an unusual pattern of developmental expression was observed for OATP1B3. Two immunoreactive OATP1B3 bands representing a core glycosylated protein and a more highly glycosylated state were observed, with high levels of expression detected in the first three months of life and declining into the early childhood years before increasing again during the preadolescent age group (6–12 years). Furthermore, the fraction of total OATP1B3 that is highly glycosylated increased significantly with age. Although few data addressing the relationship between glycosylation of transporters and cellular localization and activity are available, early studies of NTCP ontogeny in rat revealed that although adult levels of immunoreactive NTCP protein were present at 1 day after birth, glycosylation was incomplete prior to 4 weeks PNA, and more importantly, full functional activity was not observed until glycosylation was complete (Hardikar et al., 1995). Given that plasma membrane localization is also essential for functional transport activity (Schwarz et al., 2011), it is important that the data to be used for PBPK purposes most accurately reflect the developmental trajectory of functional protein, where possible. More specifically, use of plasma membrane fractions as in this study, rather than cellular lysates,

ensures that estimates of functionally active (glycosylated) transporter are not confounded by inclusion of intracellular protein that is not located in the appropriate cellular compartment.

These data on hepatic OATP expression are complemented by a proteomic analysis of hepatic transporters expression by Mooij et al. (2016) that focuses on fetal ($n = 10$) and preterm and term infantile ($n = 12$) samples. Using a liquid chromatography–tandem mass spectrometry approach after trypsin digestion of membrane preparations, Mooij et al. (2016) quantified the expression of 10 transporters, including ABCB1, ABCG2, ABCC2, ABCC3, BSEP, GLUT1, MCT1, OATP1B1, OATP2B1, and OCTN2. In this small sample with high between-patient variability, statistically significant age effects were limited to BSEP, ABCG2, GLUT1, and MCT1. Nevertheless, four different patterns of age-associated expression in the first 3 months of life were suggested by the data: 1) Stable expression for ABCB1, ABCC2, OATP1B1, and OATP2B1; 2) Low expression in the fetus that increased in the infant for ABCC3 and BSEP; 3) the inverse pattern for ABCG2, GLUT1, and OCTN2; and 4) a nonlinear relationship for MCT1. While these observations for OATP1B1 are in agreement with the data by Thomson et al. (2016), further studies will need to confirm these data for the other transporters and integrate and/or expand it with measurements in older pediatric patients and adults. Comparison with previously reported mRNA expression data on transporter maturation indicated a lack of correlation between protein and mRNA data for at least ABCB1, ABCG2, OATP1B1, and OATP2B1, an observation that has been reported previously (Ulvestad et al., 2013). This again underlines the importance of the assessment of functionally relevant moieties in the characterization of the ontogeny of transport proteins.

While knowledge about the ontogeny of transport protein expression in the liver is slowly emerging, much less is still known about maturation of transporter expression in the intestine (Brouwer et al., 2015). In a second paper, Mooij et al. (2016) investigate the mRNA expression and localization of the oligopeptide transporter PEPT1 in largely jejunal and ileal tissue specimens from preterm and term neonates and infants ($n = 20$). mRNA expression was found to be present at birth and relatively constant thereafter, with little if any change compared with a limited number of older children and adolescents. Immunohistochemistry confirmed protein expression at the apical membrane in the brush border of the enterocytes, with high but variable expression in all neonatal and infantile samples. These observations suggest that the intestinal uptake of PEPT1 substrates may not be expected to be affected by age and maturational processes.

Considered together, the papers describing the developmental trajectories of various DMEs and transporters raise several issues worthy of comment. First, the number of pediatric samples included in these investigations varies widely, ranging from approximately a dozen to over 150 samples. Furthermore, some studies with relatively small sample sets targeted an age range under-represented in ontogeny studies (for example, the first month or year of life), whereas for other studies the limited number of samples was distributed over the full pediatric age range (birth to 18 years of age), with the possibility that not all age ranges or developmental stages were adequately represented by the sample set. A second issue, which arises as a direct consequence of sample size, is how the independent variable, age, is treated during data analysis: as a continuous variable or as a categorical variable in which samples are grouped into predetermined age groups to provide sample sizes sufficient to facilitate statistical analysis.

Nested within each method of addressing age are additional issues to be considered. For example, the age of samples obtained early after birth can be expressed in terms of gestational age, PNA, or postmenstrual age (PMA). Gestational age refers to the time interval between the first day of the last regular menstrual period and the day the infant is born, measured in weeks, whereas PNA (also referred to as chronological age) represents the time elapsed after birth, and is presented in terms of days, weeks, months, or years. PMA refers to the sum of time period between the first day of the last menstrual period and birth (gestational age) plus the time after birth (PNA), and is also measured in weeks. Some studies may report the age of a newborn in terms of postconceptional or postconceptual age, but use of the term is discouraged by the American Academy of Pediatrics Committee on Fetus and Newborn (Engle et al., 2004). For investigations focusing on the first month of life, in particular, presenting the developmental trajectories as a function of PMA or PNA has the potential to confound data interpretation, especially for genes that are expressed to a limited extent during prenatal life but increase rapidly after birth. For example, available data indicate that birth is an important trigger of postnatal gene expression (Hines, 2008) and a preterm infant born at a gestational age of 35 weeks who is 5 weeks old has the same PMA, 40 weeks, as a full term infant at the time of birth (40 weeks). However, all other factors being equal, the preterm infant would be expected to exhibit higher expression of DMEs such as CYP2D6 and CYP2C19 by virtue of being 5 weeks old (PNA). Thus, the potential for the birth process as the initiating event for the onset of gene expression may be obscured when age is expressed as PMA, but will be readily apparent when age is expressed as PNA. Conversely, PNA may be misleading if the gene expression is a function of and driven by developmental (prenatal plus postnatal) age of the organism (i.e., PMA) rather than the birth process. Inconsistencies in age terminology also exist at the other end of the pediatric age spectrum. According to the National Institutes of Health (NIH) guidelines (https://www.nichd.nih.gov/health/clinicalresearch/clinical-researchers/terminology/PublishingImages/Child_Life_Stages.jpg), 21 years of age is the threshold defining pediatric or adult populations, where anyone under the age of 21 years is considered a child; in contrast, the World Health Organization (http://archives.who.int/eml/expcom/children/Items/PositionPaperAgeGroups.pdf) and the U.S. Food and Drug Administration (http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm425885.pdf and https://www.gpo.gov/fdsys/pkg/FR-1994-12-13/html/94-30238.htm) generally categorize children as 18 years or under.

Stratification of samples as prenatal, pediatric, or adult allows for broad generalizations concerning the similarity or dissimilarity of DME/transporter expression and activity between children and adults; however, data presented in such a manner should be interpreted with caution. First, if one age group is disproportionately represented in the pediatric group, such as adolescents, one may conclude that age-related differences in activity are not present when, in fact, activity may be lower (or higher) in younger age groups. Furthermore, when the pediatric group contains samples that vary in age from a few days PNA to 18 years of age, considerable variability in expression or activity may be present, often exceeding the variability observed in a set of adult samples of comparable sample size, precluding detection of real age-related effects within the pediatric group that may become evident when age is treated as a continuous variable. One approach is to apply finer stratification criteria to bin pediatric samples into categories that more or less reflect different developmental stages. For example, investigations involving relatively small sample sizes may arbitrarily select ages of 10 or 12 years to dichotomize pediatric into pre- or postpubertal groups. To improve the comparison of data across pediatric studies, for in vitro drug biotransformation and transport studies as well as in vivo PK studies, the use of a common terminology is preferred; one recommendation is the age stratification and characterization provided by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), which defines six distinct age stages as infancy (birth to 12 months), toddler (13 months to 2 years), early childhood (2–5 years), middle childhood (6–11 years), early adolescence (12–18 years), and

late adolescence (19–21 years) (Williams et al., 2012). Since considerable change in expression of DMEs and transporters may occur within the first year of life (infancy stage), finer age stratification may be preferred, in which the neonatal stage is defined as the first 28 days of postnatal life and infancy is defined as 1 month to 12 months of age.

Treating age as a continuous variable avoids the need to define arbitrary thresholds for age stratification, but does raise additional issues related to the optimal model for characterizing the changes in DME/transporter expression or activity with increasing age across the pediatric spectrum. Linear regression may be an acceptable approach for limited age ranges (for example, the first month of life or within an age stratum), but developmental changes between birth and adulthood are likely not best characterized by a linear function since continuously increasing expression with age (through to geriatrics) is implied; a similar concern could be expressed for exponential functions. Of the two groups with access to relatively large sample sizes, Pearce et al. (2016) applied a discontinuous regression model to determine that CYP2B6 expression approaches adult values by 1 year of age, whereas Hines et al. (2016) used a regression tree approach to define age strata differing with respect to CES1 and CES2 expression. Alternatives are asymptotic exponential or sigmoid E_{max} values with Hill coefficient models (Anderson and Holford, 2008), with the latter commonly used for ontogeny functions in commercial PBPK platforms.

One common feature of the studies of DME and transporter ontogeny is the source of tissues used for the investigations. Publically supported tissue repositories include the NIH-funded University of Maryland Brain and Tissue Bank for Developmental Disorders (Baltimore, MD; funded by NIH Contract HHSN275200900011C, Reference No. N01-HD-9-0011) and The Liver Tissue Cell Distribution System (funded by NIH Contract No. N01-DK-7-0004/HHSN267200700004C); prenatal tissue samples are accessible through the NICHD-funded Central Laboratory for Human Embryology at the University of Washington (Seattle, WA; 2R24HD000836-47). Pediatric tissues are also occasionally available from commercial sources, such as Cellz Direct (Carlsbad, CA), PuraCyp (Carlsbad, CA), and Xenotech (Lenexa, KS), or biorepository sources (National Disease Research Interchange, Bethesda, MD). Tissue quality is an important consideration when characterizing the developmental trajectory of DME/transporter expression or activity since poor sample quality may be responsible for low-level expression, especially early after birth. Unfortunately, no consistent approach to assessment of sample quality was applied in the ontogeny investigations included in this special issue, in large part because one does not exist. The post-mortem interval is a commonly used metric, but is difficult to apply across all tissue sources since sample quality may be quite different for tissues with a post-mortem interval of 24 hours obtained at autopsy (i.e., post-mortem tissue from the University of Maryland Brain and Tissue Bank) and those with post-mortem intervals of >24 hours but maintained in preservation buffer in anticipation of potential transplant (i.e., living donors from The Liver Tissue Cell Distribution System at the University of Pittsburgh, Pittsburgh, PA, and now at the University of Minnesota, Minneapolis, MN). Quantitative metrics of RNA quality, such as the RNA integrity number or RNA quality indicator, may prove to be of value for assessing the contribution of tissue quality to age-dependent changes in DME/transporter protein expression and activity, in addition to mRNA expression. Setting a minimum RNA integrity number or quality indicator value as a criterion for sample inclusion and conducting regression analyses on data collected are two possibilities, but certainly any contribution of sample quality should be addressed before definitive conclusions of age-dependent changes in expression/activity are made. Furthermore, there is the possibility that some DMEs and transporters may be more susceptible to intracellular degradation, which may be detected using a regression approach. Similarly, since DME/transporter expression may be low in a given sample due to genetic variation, genotype data should also be provided. Finally, liver tissue is rarely, if ever, available from normal, healthy neonates and infants shortly after birth when dynamic changes in DME and transporter expression are most pronounced. Thus, one must also take into consideration the contribution of the disease process that has resulted in the tissue becoming available in the first place.

Given that the NIH-funded tissue distribution sources assign identification numbers to the samples they distribute and multiple investigators may be using aliquots of the same tissue samples in their investigations, creation of a central repository to house the various types of DME/transporter expression and activity data generated would facilitate dissemination to the broader scientific community. One recommendation is the NICHD Data and Specimen Hub (https://dash.nichd.nih.gov/), a centralized resource created for researchers to store and access data from NICHD-funded research, although consideration should be given to be inclusive for data from non-NICHD supported studies.

Mechanisms of Developmental Regulation of DMEs and Transporters

Beyond the descriptive analysis of DME/transporter ontogeny, the mechanism(s) driving the observed developmental trajectories is also of considerable interest. Two papers provide additional insights into the well-characterized CYP3A7-CYP3A4 developmental transition that occurs between prenatal and postnatal liver. Vyhlidal et al. (2016) used methylation-sequencing analysis and confirmatory bisulfite sequencing to compare the methylation state of the *CYP3A4* and *CYP3A7* proximal promoters. Hypomethylation of CpG dinucleotides in the *CYP3A7* proximal promoter of neonatal samples compared with adults, and hypermethylation of cytosine 383 nucleotides upstream of *CYP3A4* in neonates relative to adolescent samples is consistent with the CYP3A7-CYP3A4 switch characteristic of the *CYP3A* locus after birth. Using a chromatin immunoprecipitation approach, Giebel et al. (2016) demonstrated that in prenatal liver there is significantly greater occupancy of *CYP3A4* regulatory regions with modified histones associated with repressed transcription, whereas greater occupancy by modified histones associated with active transcription was apparent in postnatal liver. The inability to generate conclusive data regarding chromatin structural dynamics and *CYP3A7* transcription in prenatal liver was attributed to cellular heterogeneity. Indeed, Vyhlidal et al. (2016) observed cytosine methylation in the *CYP3A7* proximal promoter to be similar between prenatal and adult liver despite higher CYP3A7 expression in the former; since CYP3A7 is not expressed in hematopoietic stem cells, the predominance of that cell type in human prenatal liver obscures CpG methylation in the parenchymal hepatocytes where CYP3A7 is expressed.

Since the majority of studies have characterized the developmental trajectories of basal DME and transporter expression, Li et al. (2016) reported the results of a study investigating age-specific regulation by the prototypic aryl hydrocarbon receptor, constitutive androstane receptor, and pregnane X receptor (PXR) ligands 2,3,7,8-tetrachlorodibenzodioxin, 1,4-bis [2-(3,5-dichloropyridyloxy)] benzene, and pregnane 16α-carbonitrile, respectively. Basal and induced expression of a comprehensive set (n = 29) of phase 1 and 2 DMEs, transporters, and transcription factors were assessed in C57BL/6 mice at four PNAs representing distinct developmental stages. Each prototypic ligand induced distinct sets of genes at different developmental stages. For example, Gstm2 and Gstm3 showed the greatest fold increase in mRNA expression in response to pregnane 16α-carbonitrile in the neonatal period, whereas expression of Gsta1, Gsta4, Sult1e1, Ugt1a1, Mrp3, and Mrp4 increased most in adolescent

mice, and pregnane 16α-carbonitrile–induced expression was greatest for Papss2 and Oatp1a4 in adult mice. Demonstration of age-specific patterns of gene expression in response to inducers in children would add yet another level of complexity for pharmacotherapy in pediatric patients.

Regulation of developmental and tissue-specific expression of a variety of drug transporters via hepatocyte nuclear factors Hnf1a and Hnf4a is described by Martovetsky et al. (2016). Expression of Hnf1a and Hnf4a in mouse embryonic fibroblasts as well as primary mouse proximal tubule cells led to the expression of kidney proximal tubule transporters such as Oat1, Mate1, Urat1, Bcrp, Mrp2, and Mrp4, as well as a variety of nutrient transporters and tight junction proteins. In contrast, coexpression of GATA binding protein 4 as well as forkhead box transcription factors Foxa2 and Foxa3 with Hnf1a and Hnf4a in the same cells resulted in downregulation of the proximal tubule markers and upregulation of hepatocyte marker proteins. These data support the foundational role of Hnf1a and Hnf4a as transcriptional regulators, but indicate that they are not limited to hepatocyte development and maturation and that the modulation of their effect by Gata4 and Foxa2/Foxa3 determines transdifferentiation toward either hepatocyte-like or proximal tubule-like cells. This intriguing snapshot of developmental regulation impressively demonstrates the complexity of organ and tissue-specific maturation processes.

Functional Consequences of DME and Transporter Ontogeny In Vitro

Xenobiotic exposure during growth and development may be associated with consequences that are not readily apparent from adult data due to developmental differences in the expression of DMEs and transporters, especially in the case of prenatal exposures. Given the association between in utero exposure to the environmental toxicant bisphenol A (BPA) and adverse reproductive, behavioral, metabolic, and neurologic outcomes in rodent studies, Moscovitz et al. (2016) investigated the relationship between BPA concentrations and transporter gene expression in a panel of human prenatal liver tissues ($n = 49$). BPA, free and conjugated, was detected in a significant number of prenatal liver samples, and increasing concentrations of conjugated BPA correlated significantly with expression of the transcription factor NRF2, the NRF2 target, NQO1, and the transporters MRP1, MRP2, and MRP3. The data imply that upregulation of transporters in prenatal liver may represent an adaptive response to in utero exposure to environmental contaminants; however, further investigation will be necessary to determine if the inability to adapt appropriately to toxicant challenge is associated with adverse postnatal outcomes.

Since PXR expression has been reported to be decreased in animal models of inflammatory bowel disease, Shakhnovich et al. (2016) investigated PXR expression in archived intestinal biopsies from 18 children with Crohn's disease and 12 age- and sex-matched controls aged 7–17 years of age. Expression of PXR, the PXR target CYP3A4, as well as villin, a marker of intestinal epithelial integrity, was decreased in actively inflamed small intestinal tissue (terminal ileum) but not in unaffected duodenal tissue in children with Crohn's disease, or in either tissue in children without Crohn's disease. Thus, the presence of intestinal inflammation has the potential to influence expression of genes important to drug disposition and response in children, and although archived biopsy samples are limited and preclude more comprehensive analyses, the results of this study provided support for a larger prospective investigation that is currently underway.

Use of pediatric tissues for drug biotransformation studies offers considerable potential for the development of bottom-up PBPK models to inform drug dosing in children compared with studies using adult samples. Following up on the results of a PK study of atomoxetine in children with attention-deficit/hyperactivity disorder (Brown et al., 2016), Dinh et al. (2016) characterized the P540 enzymes responsible for 4-hydroxylation in CYP2D6 poor metabolizers as well as the formation of the 2′-methylhydroxyl metabolite, which was observed to account for a greater proportion of atomoxetine biotransformation in the presence of decreased CYP2D6 activity. CYP2E1 and CYP3A were determined to contribute to 4′-hydroxy atomoxetine formation in livers with CYP2D6 intermediate and poor metabolizer status, and CYP2B6 was primarily responsible for 2′-methylhydroxy atomoxetine formation. Furthermore, a considerable amount of *N*-desmethyl atomoxetine formation was apparent in liver microsomes with the *CYP2C19* poor metabolizer genotype, which could be attributed to CYP2C18. Further characterization in a panel of pediatric liver microsomes ($n = 116$) revealed that the relative contribution of the *N*-demethylation and 2′-methylhydroxylation pathways increased as CYP2D6 activity decreased, but did not compensate for the loss of CYP2D6 activity associated with poor and intermediate metabolizer genotypes. It is anticipated that the use of pediatric microsomes to estimate intrinsic clearance of all pathways contributing to the three primary atomoxetine biotransformation pathways as well as the application of ontogeny functions to describe the changes in intrinsic clearance during growth and development will lead to the development of a PBPK model that will be applied in a future study to individualize atomoxetine doses to achieve a common systemic exposure to investigate the role of genetic variation in the drug target as a factor contributing to inter-individual variability in drug response. Intrinsic clearance is estimated using K_m and V_{max} values generated using pediatric liver microsomes, and the ontogeny function is applied to characterize changes in intrinsic clearance during growth and development.

Ontogeny of Physiologic Functions and Pharmacokinetic Processes for PBPK Modeling

Aside from enzymatic and transport processes, detailed knowledge of the age-associated changes in physiologic processes relevant for drug disposition are crucial elements necessary to facilitate the development of age-appropriate PBPK models with satisfactory predictive power. A prerequisite to capturing absorption processes after oral administration, and enterohepatic recycling processes as well, is an in-depth understanding of the small intestinal transit time and its age-associated variation. Intestinal transit time determines the contact time between a drug and the small intestinal epithelium and may thus affect the extent of its absorption, i.e., bioavailability. Maharaj and Edginton (2016) performed a random-effect meta-regression analysis with between-study variability to investigate the relationship between age and small intestinal transit time based on an extensive search of the primary literature. Their analysis included data from lactulose H_2 breath tests, scintigraphy, and other diagnostic methodologies, and indicated that in healthy subjects age does not significantly influence small intestinal transit time. Furthermore, in a subsequent PBPK modeling–based investigation for a sustained-release theophylline preparation in children between 8 and 14 years, Maharaj and Edginton (2016) showed that even large alterations (−50%) of the small intestinal transit time lacked influence on the oral absorption of sustained-release theophylline. Discernable changes were only observed when the total intestinal transit time was greatly altered. Since theophylline is a Biopharmaceutics Classification System class I drug (high solubility and high permeability), it remains to be seen whether these observations would also hold true for compounds with limited solubility and/or permeability.

Similarly, Johnson et al. (2016) investigated the association between age and biliary excretion based on the published literature. However, in contrast to Maharaj and Edginton (2016), Johnson et al. (2016) did not

use directly measured functional data but rather used PK data on the biliary excreted drugs azithromycin, ceftriaxone, digoxin, and buprenorphine. In a reverse engineering PBPK approach, they determined the fraction of adult biliary excretion necessary to mimic the observed concentration-time profiles in pediatric patients of different ages, thereby assuming no ontogeny for the biliary excretion pathway but ontogeny for all nonbiliary elimination pathways. Their investigations suggest that, in general, biliary excretion seems to develop rapidly and be at adult equivalent capacity soon after birth. Since biliary excretion is the function of multiple transporters, particularly P-glycoprotein (ABCB1), MRP2 (ABCC2), and BCRP, and the investigated drugs are substrates for different transporters, differential effects were observed in this study and may be observed for other substrates. Nevertheless, this approach impressively illustrates how PBPK modeling combined with clinical PK data can be integrated to assess unknown system parameters such as the age-associated changes in biliary clearance.

Given that the unbound fraction of drug in blood, hepatic clearance of unbound drug, and hepatic blood flow are all age-dependent parameters and are determinants of the hepatic extraction (E_H) ratio, Salem et al. (2016) investigated the potential for disproportionate age-related changes in these components to result in age-dependent assignment of a drug as a low, intermediate, or high extraction compound. PBPK-based simulations using midazolam and two hypothetical compounds with 10-fold higher and 10-fold lower values in hepatic clearance of unchanged drug model compounds, midazolam E_H increased from 0.02 at birth to 0.6 in adults; the low clearance compound remained a low extraction drug throughout the entire age range, whereas the high clearance compound was classified as a high extraction drug after 4 days of age. The results of this analysis indicate that E_H cannot be considered an inherent drug property, and pediatric simulations based on adult E_H data should consider potential effects of age. Likewise, the recent report of age-dependent changes in plasma protein binding patterns for diazepam, cyclosporine, and the pesticide deltamethrin (Sethi et al., 2016) indicates that concerted efforts to obtain pediatric data wherever possible should be preferred over a default position of extrapolation from adults.

Functional Consequences of Ontogeny and Genetic Variation In Vivo

The application of PBPK modeling to describe and predict drug disposition in pediatric patients based on clinical observations in adults may further be complicated if other intrinsic factors beyond size, physiology, and ontogeny of enzymatic and transport processes need to be scaled from adults to pediatric patients. Rasool et al. (2016) demonstrated this challenge by applying a full body PBPK model for the stereoselective disposition of carvedilol in patients with chronic heart failure. While the hemodynamic changes associated with chronic heart failure in adult patients require organ blood flow reductions to adequately describe the PK of the enantiomers of the hepatic high extraction drug carvedilol, pediatric patients under 12 years of age do not seem to require these flow reductions in the model. This observation suggests that disease-specific processes relevant to drug disposition such as organ blood flow in chronic heart failure patients cannot necessarily be directly scaled to the pediatric situation without careful case-by-case evaluation.

Orally administered cancer treatments are associated with considerable inter- and intrasubject variability. Variability in the bioavailability of orally administered medication is even greater in infants and young children due to developmental changes in factors affecting drug absorption, such as higher gastric pH and slower gastric motility. An additional source of variability is genetic variation; however, until the gene is expressed to a significant extent it is unlikely that pharmacogenetic influences can be detected. In this special issue, Roberts et al. (2016) report the results of their investigation of the effect of genetic variation in *ABCB1* and *ABCG2* on the pharmacokinetics of orally administered topotecan lactone in 61 young children between 0.5 and 4.5 years of age, 20 of whom were less than 2 years of age. Using a population PK approach, genotypes containing the minor A allele of *ABCG2* rs4148157 were associated with a 2-fold greater absorption rate constant relative to the homozygous GG genotype. These results imply that *ABCG2* expression has matured sufficiently for a pharmacogenetic effect to be detected in young children, and are also consistent with early onset of functional ABCG2 activity implied by the time course for maturation of biliary clearance presented by Johnson et al. (2016), as described previously. Furthermore, this study makes a case for the importance of accurate, well-characterized ontogeny functions for DMEs and transporters such that additional patient-related factors (e.g., genotype) can be appropriately considered in predictive models.

Summary

It is generally acknowledged that there is a need for informative PK models that can play a role in improving modeling and simulation as well as individualization of drug therapy in pediatric populations of different ages and at different developmental stages. This special issue contains more than 20 papers responding to the challenge of providing new information on scaling factors, ontogeny functions for DMEs and transporters, the mechanisms underlying the observed developmental trajectories for these gene products, age-dependent changes in physiologic processes affecting drug disposition in children, as well as in vitro and in vivo studies describing the relative contribution of ontogeny and genetic factors as sources of variability in drug disposition in children. Considered together, these contributions serve to illustrate some of the current limitations regarding sample availability, number, and quality; however, they also provide a framework that allows for the potential value of the results of a given study to be interpreted within the context of these limitations. The challenge for the future is to consolidate available information into more sophisticated models for simulation and individualization of drug disposition and response across the pediatric age range.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Leeder, Meibohm

References

Abduljalil K, Jamei M, Rostami-Hodjegan A, and Johnson TN (2014) Changes in individual drug-independent system parameters during virtual paediatric pharmacokinetic trials: introducing time-varying physiology into a paediatric PBPK model. *AAPS J* **16**:568–576.

Anderson BJ and Holford NHG (2008) Mechanism-based concepts of size and maturity in pharmacokinetics. *Annu Rev Pharmacol Toxicol* **48**:303–332.

Anderson BJ and Holford NHG (2013) Understanding dosing: children are small adults, neonates are immature children. *Arch Dis Child* **98**:737–744.

Barrett JS, Della Casa Alberighi O, Läer S, and Meibohm B (2012) Physiologically based pharmacokinetic (PBPK) modeling in children. *Clin Pharmacol Ther* **92**:40–49.

Barter ZE, Bayliss MK, Beaune PH, Boobis AR, Carlile DJ, Edwards RJ, Houston JB, Lake BG, Lipscomb JC, and Pelkonen OR, et al. (2007) Scaling factors for the extrapolation of in vivo metabolic drug clearance from in vitro data: reaching a consensus on values of human microsomal protein and hepatocellularity per gram of liver. *Curr Drug Metab* **8**:33–45.

Barter ZE, Chowdry JE, Harlow JR, Snawder JE, Lipscomb JC, and Rostami-Hodjegan A (2008) Covariation of human microsomal protein per gram of liver with age: absence of influence of operator and sample storage may justify interlaboratory data pooling. *Drug Metab Dispos* **36**:2405–2409.

Brouwer KL, Aleksunes LM, Brandys B, Giacoia GP, Knipp G, Lukacova V, Meibohm B, Nigam SK, Rieder M, and de Wildt SN; Pediatric Transporter Working Group (2015) Human ontogeny of drug transporters: Review and recommendations of the Pediatric Transporter Working Group. *Clin Pharmacol Ther* **98**:266–287.

Brown JT, Abdel-Rahman SM, van Haandel L, Gaedigk A, Lin YS, and Leeder JS (2016) Single dose, CYP2D6 genotype-stratified pharmacokinetic study of atomoxetine in children with ADHD. *Clin Pharmacol Ther* **99**:642–650.

Cubitt HE, Houston JB, and Galetin A (2011) Prediction of human drug clearance by multiple metabolic pathways: integration of hepatic and intestinal microsomal and cytosolic data. *Drug Metab Dispos* **39**:864–873.

Chen Y, Zane NR, Thakker DR, and Wang MZ (2016) Quantification of flavin-containing monooxygenases 1, 3, and 5 in human liver microsomes by UPLC-MRM-based targeted quantitative proteomics and its application to the study of ontogeny. *Drug Metab Dispos* **44**:975–983.

Dinh JC, Pearce RE, Haandel LV, Gaedigk A, and Leeder JS (2016) Characterization of atomoxetine biotransformation and implications for development of PBPK models for dose individualization in children. *Drug Metab Dispos* **44**:1070–1079.

Edginton AN and Willmann S (2006) Physiology-based versus allometric scaling of clearance in children: an eliminating process-based comparison. *Paediatr Perinat Drug Ther* **7**: 146–153.

Engle WA; American Academy of Pediatrics Committee on Fetus and Newborn (2004) Age terminology during the perinatal period. *Pediatrics* **114**:1362–1364.

Elmorsi Y, Barber J, and Rostami-Hodjegan A (2016) Ontogeny of hepatic drug transporters and relevance to drugs used in paediatrics. *Drug Metab Dispos* **44**:992–998.

Fattah S, Augustijns P, and Annaert PP (2016) Effect of age on the hepatocellularity number for Wistar rats. *Drug Metab Dispos* **44**:944–947.

Giebel NL, Shadley JD, McCarver DG, Dorko K, Gramignoli R, Strom SC, Yan K, Simpson PM, and Hines RN (2016) Role of chromatin structural changes in regulating human CYP3A octogeny. *Drug Metab Dispos* **44**:1027–1037.

Hardikar W, Ananthanarayanan M, and Suchy FJ (1995) Differential ontogenic regulation of basolateral and canalicular bile acid transport proteins in rat liver. *J Biol Chem* **270**: 20841–20846.

Hines RN (2008) The ontogeny of drug metabolism enzymes and implications for adverse drug events. *Pharmacol Ther* **118**:250–267.

Hines RN, Simpson PM, and McCarver DG (2016) Age-dependent human hepatic carboxylesterase1 (CES1) and carboxylesterase 2 (CES2) postnatal ontogeny. *Drug Metab Dispos* **44**: 959–966.

Johnson TN, Jamei M, and Rowland-Yeo K (2016) How does the in vivo biliary elimination of drugs change with age? Evidence from in vitro and clinical data using a systems pharmacology approach. *Drug Metab Dispos* **44**:1090–1098.

Johnson TN and Rostami-Hodjegan A (2011) Resurgence in the use of physiologically based pharmacokinetic models in pediatric clinical pharmacology: parallel shift in incorporating the knowledge of biological elements and increased applicability to drug development and clinical practice. *Paediatr Anaesth* **21**:291–301.

Läer S, Barrett JS, and Meibohm B (2009) The in silico child: using simulation to guide pediatric drug development and manage pediatric pharmacotherapy. *J Clin Pharmacol* **49**:889–904.

Li CY, Renaud HJ, Klaassen CD, and Cui JY (2016) Age-specific regulation of drug-processing genes in mouse liver by ligans of xenobiotic-sensing transcription factors. *Drug Metab Dispos* **44**:1038–1049.

Maharaj AR and Edginton AN (2016) Examining small intestinal transit time as a function of age – is there evidence to support age-dependent differences among children? *Drug Metab Dispos* **44**: 1080–1089.

Martovetsky G, Bush KT, and Nigam SK (2016) Transcription factor-mediated regulation of kidney versus liver specification of Slc and Abc drug transporters, tight junction molecules and signature biomarkers. *Drug Metab Dispos* **44**:1050–1060.

Mehrotra N, Bhattaram A, Earp JC, Florian J, Krudys K, Lee JE, Lee JY, Liu J, Mulugeta Y, and Yu J, et al. (2016) Role of quantitative clinical pharmacology in pediatric approval and labeling. *Drug Metab Dispos* **44**:924–933.

Mooij MG, van de Steeg E, Rosmalen JV, Windster JD, de Koning BAE, Vaes WH, van Groen BD, Tibbuel D, Wortelboer HM, and de Wildt SN (2016) Proteomic analysis of the developmental trajectory of human hepatic membrane transporter proteins in the first three months of life. *Drug Metab Dispos* **44**:1005–1013.

Mooij MG, de Koning BAE, Lindenbergh-Kortleve DJ, Simons-Oosterhuis T, van Groen BD, Tibboel D, Samsom JN, and de Wildt SN (2016) Human intestinal PEPT1 transporter expression and localization in preterm and term infants. *Drug Metab Dispos* **44**:1014–1019.

Moscovitz JE, Nahar MS, Shalat SL, Slitt AL, Dolinoy DC, and Aleksunes LM (2016) Correlation between conjugated bisphenol A concentrations and efflux transporter expression in human fetal livers. *Drug Metab Dispos* **44**:1061–1065.

Pearce RE, Gaedigk R, Twist GP, Dai H, Riffel AK, Leeder S, and Gaedigk A (2016) Developmental expression of CYP2B6: a comprehensive analysis of mRNA expression, protein content and bupropion hydroxylase activity and the impact of genetic variation. *Drug Metab Dispos* **44**:948–958.

Rasool MF, Khalil F, and Laer S (2016) Predicting stereo-selective disposition of carvedilol in adult and pediatric chronic heart failure patients by incorporating pathophysiological changes in organ blood flows – a physiologically based pharmacokinetic approach. *Drug Metab Dispos* **44**: 1103–1115.

Rioux N and Waters NJ (2016) Physiologically-based pharmacokinetic modeling in pediatric oncology drug development. *Drug Metab Dispos* **44**:934–943.

Roberts JK, Birg AV, Lin T, Daryani VM, Panetta JC, Broniscer A, Robinson GW, Gajjar AJ, and Stewart CF (2016) Population pharmacokinetics of oral topotecan in infants and very young children with brain tumors demonstrates a role of ABCG2 rs4148157 on the absorption rate constant. *Drug Metab Dispos* **44**:1116–1122.

Rougee LRA, Riches Z, Berman JM, and Collier AC (2016) The ontogeny and population variability of human hepatic NAD(P)H dehydrogenase quinone oxido-reductase 1 (NQO1). *Drug Metab Dispos* **44**:967–974.

Rostami-Hodjegan A and Tucker GT (2007) Simulation and prediction of in vivo drug metabolism in human populations from in vitro data. *Nat Rev Drug Discov* **6**:140–148.

Sadler NC, Nandhikonda P, Webb-Robertson BJ, Ansong C, Anderson LN, Smith JN, Corley RA, and Wright AT (2016) Hepatic cytochrome P450 activity, abundance, and expression throughout human development. *Drug Metab Dispos* **44**:984–991.

Salem F, Abduljalil K, Kamiyama Y, and Rostami-Hodjegan A (2016) Considering age variation when coining drugs as high vs low hepatic extraction ratio. *Drug Metab Dispos* **44**:1099–1102.

Samant TS, Mangal N, Lukacova V, and Schmidt S (2015) Quantitative clinical pharmacology for size and age scaling in pediatric drug development: A systematic review. *J Clin Pharmacol* **55**: 1207–1217.

Schwarz UI, Meyer zu Schwabedissen HE, Tirona RG, Suzuki A, Leake BF, Mokrab Y, Mizuguchi K, Ho RH, and Kim RB (2011) Identification of novel functional organic anion-transporting polypeptide 1B3 polymorphisms and assessment of substrate specificity. *Pharmacogenet Genomics* **21**:103–114.

Sethi PK, White CA, Cummings BS, Hines RN, Muralidhara S, and Bruckner JV (2016) Ontogeny of plasma proteins, albumin and binding of diazepam, cyclosporine, and deltamethrin. *Pediatr Res* **79**:409–415.

Shakhnovich V, Vyhlidal CA, Friesen C, Hildreth A, Singh V, Daniel J, Kearns GL, and Leeder JS (2016) Decreased pregnane X receptor (PXR) expression in children with active Crohn's disease. *Drug Metab Dispos* **44**:1066–1069.

Thomson MMS, Hines RN, Schuetz EG, and Meibohm B (2016) Expression patterns of OATP 1B1 and OATP 1B3 protein in human pediatric liver. *Drug Metab Dispos* **44**:999–1004

Tsamandouras N, Rostami-Hodjegan A, and Aarons L (2015) Combining the 'bottom up' and 'top down' approaches in pharmacokinetic modelling: fitting PBPK models to observed clinical data. *Br J Clin Pharmacol* **79**:48–55.

Ulvestad M, Skottheim IB, Jakobsen GS, Bremer S, Molden E, Asberg A, Hjelmesæth J, Andersson TB, Sandbu R, and Christensen H (2013) Impact of OATP1B1, MDR1, and CYP3A4 expression in liver and intestine on interpatient pharmacokinetic variability of atorvastatin in obese subjects. *Clin Pharmacol Ther* **93**:275–282.

Vinks AA, Emoto C, and Fukuda T (2015) Modeling and simulation in pediatric drug therapy: Application of pharmacometrics to define the right dose for children. *Clin Pharmacol Ther* **98**:298–308.

Vyhlidal CA, Bi C, Ye SQ, and Leeder JS (2016) Dynamics of cytosine methylation in the proximal promoters of CYP3A4 and CYP3A7 in pediatric and prenatal liver. *Drug Metab Dispos* **44**:1020–1026.

Williams K, Thomson D, Seto I, Contopoulos-Ioannidis DG, Ioannidis JPA, Curtis S, Constantin E, Batmanabane G, Hartling L, and Klassen T; StaR Child Health Group (2012) Standard 6: age groups for pediatric trials. *Pediatrics* **129** (Suppl 3):S153–S160.

Zhang H, Gao N, Tian X, Liu T, Fang Y, Zhou J, Wen Q, Xu B, Qi B, and Gao J, et al. (2015) Content and activity of human liver microsomal protein and prediction of individual hepatic clearance in vivo. *Sci Rep* **5**:17671.

Address correspondence to: Dr. J. Steven Leeder, Division of Clinical Pharmacology, Toxicology and Therapeutic Innovation, Department of Pediatrics, Children's Mercy Hospitals and Clinics, 2401 Gillham Road, Kansas City, MO 64108. E-mail: sleeder@cmh.edu