

Impact of developmental pharmacology on pediatric study design: Overcoming the challenges

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The need to establish drug-dosing guidelines in children highlights the challenges associated with the development of phases I and II pediatric clinical trials. These challenges are the consequence of significant developmental changes that characterize childhood and adolescence and can affect drug absorption, binding, renal elimination, and, especially, metabolism. In addition, genetic polymorphism can contribute to the variations in the expression of activity for specific drug-metabolizing enzymes. These developmental and genetic variations in pharmacokinetics are the major determinants of drug exposure over time and are thus directly related to the safety, efficacy, and toxicity of a drug dose. Therefore, in the development of pediatric protocols and appropriate dosing in children, it is essential to develop a strategy for addressing the developmental variables that affect drug exposure and to incorporate them into study design. (*J Allergy Clin Immunol* 2000;106:S128-38.)

Key words: Pharmacokinetics, pharmacodynamics, cytochromes, genetic polymorphism

New federal regulations, such as the Food and Drug Administration Modernization Act, now require drug manufacturers to provide age-appropriate dosing guidelines for medications that are routinely prescribed, but not explicitly indicated, for pediatric use. Because of the paucity of studies designed to evaluate proper drug dosing in children, physicians are often placed at a distinct disadvantage when trying to make informed therapeutic decisions. Consequently, patients can be placed at increased risk for adverse drug effects.^{1,2}

With the proliferation of new chemical entities and the "off-label" use of many marketed drug products in pediatric patients, there is growing need for the performance of studies that are sufficient to define age-appropriate dosing regimens. In addition, investigations that characterize the clinical pharmacologic features of drugs in certain pediatric populations (eg, neonates, infants, and adolescents) are frequently necessary when clear differences exist between children and adults in a given disease process or when age per se may significantly alter the dose versus concentration versus effect relationship.

Abbreviations used

AUC: Area under the plasma concentration vs time curve

CYP: Cytochrome 450

S-HPPH: (S)-5-(4-hydroxyphenyl)-5-phenylhydantion

UGT: Uridine-5 -diphosphate-glucuronosyltransferase

When pediatric protocols are being developed, it is critical to recognize that infants and children cannot be viewed simply as small adults. In addition to the obvious psychologic and behavioral differences, infants and children undergo significant developmental changes in physiologic processes over relatively short periods of time. Because these changes are often the "drivers" of alterations in drug disposition, they must be considered in pediatric protocol design. I examine relevant changes in pediatric pharmacology and their implications for effective and well-tolerated pediatric dosing and will recommend specific considerations for pediatric study design.

DEVELOPMENTAL PHARMACOKINETIC CHALLENGES

During childhood, which can be broadly defined as the time from birth through puberty, rapid developmental changes occur that can have a profound effect on pharmacokinetics and pharmacodynamics. The most dramatic pharmacokinetic changes occur during the first 12 months of life and affect a wide array of processes, including absorption, protein binding, renal elimination, and drug biotransformation.

Absorption

Both the rate and extent of extravascular drug absorption may differ markedly in the neonate and young infant as compared with children and young adults. Developmental differences in hydrogen ion excretion and gastric and intestinal motility and the impact of development on the activity of drug-metabolizing enzymes located in the small intestine must be considered. The rate and extent of gastrointestinal absorption depends primarily on pH-dependent passive diffusion and the motility of the stomach and small intestine, both of which control transit time. In term (fully mature) neonates, the gastric pH ranges from 6 to 8 at birth and drops to 2 to 3 within the first few hours. After the first 24 hours of extrauterine life, the gastric pH increases to approximately 6 to 7, consequent to immaturity of the parietal cells. A relative

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0091-6749/2000 \$12.00 + 0 1/0/109419

doi:10.1067/mai.2000.109419

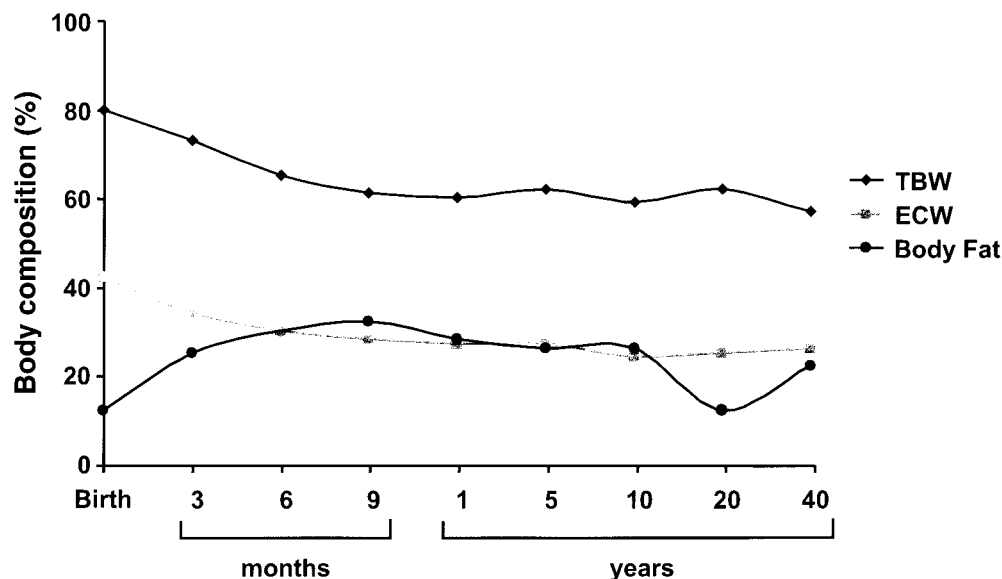


FIG 1. Alterations in the total body water (TBW), extracellular water (ECW), and body fat "pools" are illustrated. The most dynamic changes occur in the first year of life, with the exception of total body fat, which, in male patients, is reduced by approximately 50% between 10 and 20 years of life. In female patients, this reduction is not as dramatic. Data represent mean values for male patients. (From Ritschel WA, Kearns GL, editors. *Pediatric pharmacokinetics*. In: *Handbook of basic pharmacokinetics*. 5th ed. Washington, DC: American Pharmaceutical Association; 1999. p. 304-21. Reprinted by permission from the *Handbook of Basic Pharmacokinetics*, 5th edition. © 1999 by the American Pharmaceutical Association.)

state of achlorhydria remains until adult values for gastric pH are reached at 20 to 30 months of age.³

In the neonate, gastrointestinal transit time is prolonged because of reduced motility and peristalsis. Gastric emptying is both irregular and erratic, and only partially dependent on feeding. Gastric emptying rates approximate adult values by 6 to 8 months of age. During infancy, intestinal transit time is generally reduced relative to adult values consequent to increased intestinal motility. In the neonate and young infant, additional factors may play a role in intestinal drug absorption. These include relative immaturity of the intestinal mucosa leading to increased permeability, immature biliary function, high levels of intestinal β -glucuronidase activity, and variable microbial colonization.³

The developmental changes in gastrointestinal function and structure during the newborn period and early infancy produce alterations in drug absorption which, in some cases, are quite predictable. In general, the oral bioavailability of acid-labile compounds (eg, β -lactam antibiotics) is increased, although that of weak organic acids (eg, phenobarbital and phenytoin) is decreased. For orally administered drugs with limited water solubility (eg, phenytoin and carbamazepine), the rate of absorption can be dramatically altered as a result of changes in gastrointestinal motility.³ The pharmacokinetics of the antiviral agent pleconaril provide an example of the difference in absorption among adults, children, and neonates.⁴⁻⁶ After a 5-mg/kg dose, the apparent plasma clearance and volume of distribution were substantially higher in neonates (CL/F, 1.3 ± 1.2 L/h/kg; Vdss/F, 9.3 ± 6.9 L/kg, respectively) than in either children ($0.84 \pm$

0.86 L/h/kg and 4.68 ± 2.02 L/kg, respectively) or adults (0.34 ± 0.12 L/h/kg and 1.94 ± 1.54 L/kg, respectively). For neonates, the area under the plasma concentration versus the time curve (AUC) for pleconaril was much lower than that for children or adults, which highlights the fact that young children do not absorb pleconaril well. The reason for this difference may be attributed to the pleconaril lipid formulation and the developmental differences in the ability to absorb lipids consequent to reduced lipase secretion in neonates.⁶

In the newborn and young infant, both rectal and percutaneous absorption can serve as highly efficient routes of administration for properly formulated drug products. The bioavailability of many drugs administered by the rectal route (eg, diazepam, acetaminophen) may be increased in the young infant consequent to reduced presystemic clearance that, in part, is a function of developmental immaturity of hepatic drug-metabolizing enzymes. Both the rate and extent of percutaneous drug absorption is increased because of a thinner and more well-hydrated stratum corneum in the young infant. As a result, systemic toxicity can be seen with a percutaneous application of some drugs (eg, diphenhydramine, lidocaine, corticosteroids, and hexachlorophene) to seemingly small areas of the skin during the first 8 to 12 months of life. Finally, the rate of bioavailability for drugs that are administered by the intramuscular route may be altered (delayed the rate of absorption) in the neonate because of relatively low muscular blood flow in the first few days of life, the relative inefficiency of muscular contractions (useful in dispersing an intramuscular drug dose), and an increased percentage of water per unit of

TABLE I. Summary of drug absorption in neonates, infants, and children

	Neonate	Infants	Children
Physiologic alteration			
Gastric emptying time	Irregular	Increased	Slightly increased
Gastric pH	>5	4-2	Normal (2-3)
Intestinal motility	Reduced	Increased	Slightly increased
Intestinal surface area	Reduced	Near adult	Adult pattern
Biliary function	Immature	Near adult	Adult pattern
Muscular blood flow	Reduced	Increased	Adult pattern
Skin permeability	Increased	Increased	Near adult pattern
Possible pharmacokinetic consequences			
Oral absorption	Erratic-reduced	↑Rate*	Near adult pattern
Intramuscular absorption	Variable	Increased	Adult pattern
Percutaneous absorption	Increased	Increased	Near adult pattern
Rectal absorption	Very efficient	Efficient	Near adult pattern
Presystemic clearance	<Adult	>Adult	>Adult (↑ rate*)

*The direction of alteration is given relative to the expected normal adult pattern.

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muscle mass. Generally, intramuscular absorption of drugs in the neonate is much slower and more erratic with the rate, dependent on the physicochemical properties of the drug and on the maturational stage of the newborn infant.³ Age-dependent differences that impact drug absorption from extravascular routes of administration are summarized in Table I.

Drug distribution

During development, marked changes in body composition occur. Alterations in the total body water, extracellular water, and body fat "pools" are illustrated in Fig 1. The most dynamic changes occur in the first year of life with the exception of total body fat which, in male patients, is reduced by approximately 50% between 10 and 20 years of life. In female patients, this reduction is not as dramatic, decreasing from approximately 28% to 25% during this same period. It is also important to note that the neonate's adipose tissue may contain as much as 57% water and 35% lipids, whereas adult values approach 26.3% and 71.7%, respectively.³

In addition to age-related alterations in body composition, the neonatal period is characterized by several physiologic alterations that are capable of reducing the plasma protein binding of drugs (Table II). In the neonate, the free fraction of drugs that is extensively bound (>60%) to circulating plasma proteins is markedly increased, largely because of lower concentrations of drug-binding proteins (ie, a lower number of binding sites), reduced binding affinity (eg, the lower binding affinity for weak acids to fetal albumin, the presence of acidic plasma pH, and the presence of endogenous competing ligands [such as bilirubin and free fatty acids]). This is exemplified by phenytoin, a weak acid that is 94% to 98% bound to albumin in adults (free fraction, 2% to 4%) but only 80% to 85% bound in the neonate (free fraction, 15% to 20%).³ This particular age-dependent alteration in drug binding functionally reduces the total plasma phenytoin level that is

associated with both efficacy and toxicity in the newborn, as compared with older infants and children. Reduced plasma protein binding associated with absolute and relative differences in the sizes of various body compartments (eg, total body water, extracellular fluid, and composition of body tissues) frequently influences the apparent volume of distribution for many drugs and also influences their localization (both uptake and residence) in tissue.

Renal elimination

The renal excretion of many drugs is directly proportional to age-dependent patterns in the acquisition of renal function, primarily glomerular filtration and active tubular secretion. Accordingly, developmental differences in renal function may serve as a major determinant of drug clearance in the neonate and young infant.

In the preterm infant, renal function is dramatically reduced because of the continued development of functioning nephron units (nephrogenesis). In contrast, the acquisition of renal function in the term neonate represents, to a great degree, recruitment of fully developed nephron units. In both term neonates and preterm infants with birth weights of more than 1500 gm, glomerular filtration rates increase dramatically during the first 2 weeks of postnatal life (Fig 2). This particular dynamic change in function is a direct result of postnatal adaptations in the distribution of renal blood flow, which results in dramatic recruitment of functioning nephron units. In addition, there exists a glomerular/tubular imbalance because of a more advanced maturation of glomerular function, as compared with proximal tubular secretion. This imbalance may persist up to 6 to 10 months of age, at which time both tubular and glomerular function approach values approximately equal to those observed in healthy, young adults.³

The impact of development on each of the components of renal function (eg, glomerular filtration, proximal tubular secretion and reabsorption, and organic base transport) can be characterized by a definable pattern

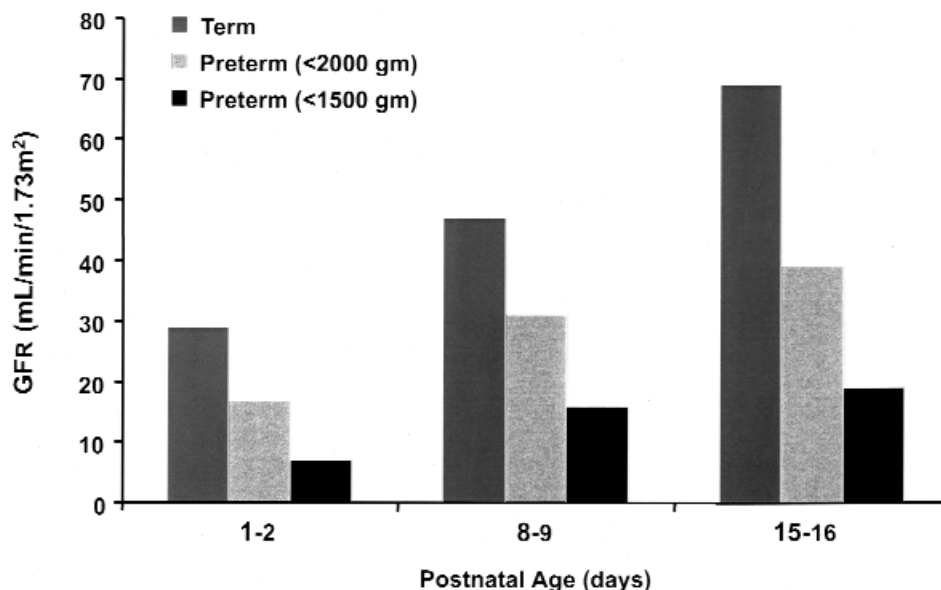


FIG 2. In both term neonates and preterm infants who have birth weights of more than 1500 gm, glomerular filtration rates increase dramatically during the first 2 weeks of postnatal life. (From Ritschel WA, Kearns GL, editors. Pediatric pharmacokinetics. In: Handbook of basic pharmacokinetics. 5th ed. Washington, DC: American Pharmaceutical Association; 1999. p. 304-21. Reprinted by permission of the Handbook of Basic Pharmacokinetics, 5th ed. © 1999 by the American Pharmaceutical Association.)

TABLE II. Plasma protein binding and drug distribution

	Neonate	Infants	Children
Physiologic alteration			
Plasma albumin	Reduced	Near normal	Near adult pattern
Fetal albumin	Present	Absent	Absent
Total proteins	Reduced	Decreased	Near adult pattern
Total globulins	Reduced	Decreased	Near adult pattern
Serum bilirubin	Increased	Normal	Normal adult pattern
Serum free fatty acids	Increased	Normal	Normal adult pattern
Blood pH	7.1-7.3	7.4 (normal)	7.4 (normal)
Adipose tissue	Scarce (↑CNS)	Reduced	Generally reduced
Possible pharmacokinetic consequences			
Free fraction	Increased	Increased	Slightly increased
Apparent volume of distribution			
Hydrophilic drugs	Increased	Increased	Slightly increased
Hydrophobic drugs	Reduced	Reduced	Slightly decreased
Tissue/plasma ratio	Increased	Increased	Slightly increased

CNS, Central nervous system.

The direction of alteration is given relative to the expected normal adult pattern.

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during the first year of life.³ Accordingly, the renal handling and the excretion/elimination characteristics of virtually any drug in neonates, infants, and children can largely be predicted by considering the ontogeny of renal function and the specific pharmacologic characteristics (eg, routes of renal excretion and the percentage of a given dose excreted unchanged in the urine) of a given drug as regards to its renal excretion in adults. As is evident from data published by James et al,^{7,8} linearity in the relationship between famotidine plasma clearance and age is predicted, given the dependence of clearance on glomerular filtration (Fig 3).

Drug metabolism

In infants, children, and adolescents, developmental variations in drug metabolism have been associated with age, gender, maturation, and genetic constitution. During specific periods of development, these factors can profoundly affect the expression of multiple enzymes that are quantitatively important in the regulation of the biotransformation of drugs. The magnitude of developmental differences in drug metabolism in many instances is sufficient to alter a given child's exposure to certain drugs and accordingly is often the most important pharmacokinetic determinant of age-appropriate dose selection.

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FIG 3. Linearity in the relationship between famotidine plasma clearance (CL) and age is predicted given the dependence of clearance on glomerular filtration. (From James LP, Marshall JD, Heullitt MJ, Wells TG, Letzig L, Kearns GL. Pharmacokinetics and pharmacodynamics of famotidine in children. *J Clin Pharmacol* 1996;36:48-54. Copyright © 1996 by Sage Publications, Inc. Reprinted by permission of Sage Publications, Inc.)

Much of the existing information concerning drug metabolism in infants, children, and adolescents has been derived as a “by-product” of pharmacokinetic investigations that were designed in part to determine whether age-dependent differences in drug disposition (eg, drug clearance) were evident. For many compounds, developmental differences in the clearance of drugs in which the primary biotransformation pathways are known have produced partial developmental “road maps,” which have shed information on the patterns of ontogeny for important drug-metabolizing enzymes.

PHASE I PATHWAYS

The use of pharmacokinetic data to examine the ontogeny of a drug-metabolizing enzyme is well illustrated by theophylline, a pharmacologic substrate for cytochrome 450 (CYP) 1A2. Nassif et al⁹ reported elimination half-life values for theophylline that ranged between 8 and 18 hours in term infants 6 to 12 weeks of postnatal age. Furthermore, these investigators found a linear relationship between postnatal age and theophylline half-life, with values declining to approximately 3 to 4 hours by 48 weeks of life. Over a decade later, Kraus et al¹⁰ demonstrated that the dramatic alterations in theophylline plasma clearance that occur between 30 weeks (approximately 10 mL/h/kg) and 100 weeks (approximately 80 mL/h/kg) of postconceptional age were primarily the result of age-dependent differences in the metabolism of theophylline by CYP1A2-dependent pathways. Further characterization of theophylline biotransformation in humans by Tjia et al¹¹ demonstrated that theophylline was adequate for use as a pharmacologic “probe” to assess CYP1A2 activity, given that approximately 80% of the formation of 1,3-dimethyluric acid at theophylline concentrations of 100 μ mol/L was cat-

alyzed by this P450 isoform. Recently, Tateishi et al¹² administered theophylline to 51 pediatric patients who ranged in age from 1 month to 14 years and examined the urinary ratios of 3 metabolites: 1-methyluric acid, 3-methylxanthine and 1,3-dimethyluric acid. Examination of the urinary ratio of 1,3-dimethyluric acid to either 3-methylxanthine or 1-methyluric acid (both generated by CYP1A2) demonstrated that adult competence of CYP1A2 activity was reached by 3 months of postnatal age, a finding that corroborated earlier pharmacokinetic studies of the drug in infants.

Although collectively these data appear to have created a well-defined pattern of CYP1A2 ontogeny, early studies by Lambert et al¹³ and a recent investigation by Gotschall et al¹⁴ demonstrated that both puberty and cystic fibrosis, respectively, influence the pattern of CYP1A2 ontogeny (reflected by the use of methylxanthines as probe compounds) and thus implicate sexual maturation and disease as potentially important covariates for the expression of this particular cytochrome P450 during development.

Another example in which pharmacokinetic data have shed important insights concerning the impact of development on drug metabolism resides with CYP3A4, which is the most abundant CYP isoform in the human body and is responsible for catalyzing the biotransformation of well over 20 drugs that are commonly used in pediatric practice.¹⁵ As recently reviewed by de Wildt et al,¹⁶ ontogeny appears to have a major impact on the activity of CYP3A4. Like the activity of CYP1A2, CYP3A4 activity appears to be greater in infants and children compared with adults. A study of carbamazepine and carbamazepine 10,11-epoxide (a CYP3A4 product) conducted in infants and children 2 weeks to 15 years of age demonstrated an age-dependent decrease in the ratio of the epoxide metabolite to the parent drug in serum.¹⁷ These data sug-

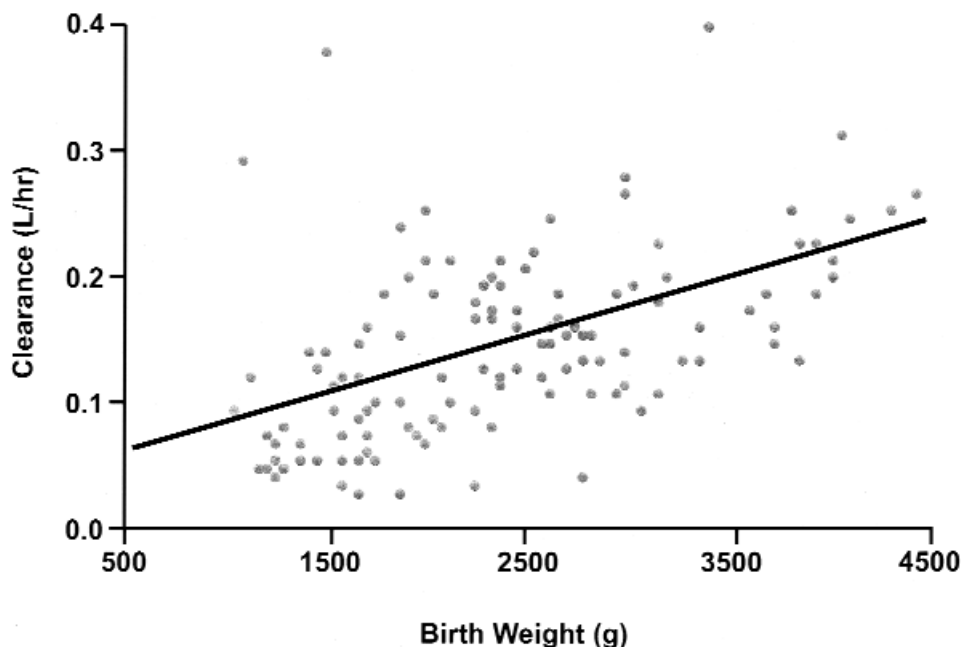


FIG 4. The uncorrected (L/h) plasma clearance of midazolam at birth is directly correlated with birth weight, a surrogate measure for CYP3A4 competence. (Adapted from Burtin P, Jacqz-Aigrain E, Girard P, Lenclen R, Magney JF, Betremieux P, et al. Population pharmacokinetics of midazolam in neonates. *Clin Pharmacol Ther* 1994;56:615-25. With permission.)

gest a higher CYP3A4 activity in children and a gradual maturation to adult levels of activity during adolescence. Variability in the activity of microsomal epoxide hydrolase, which further catalyzes the biotransformation of the 10,11-epoxide to the corresponding transdihydrodiol, may confound interpretation of these data, however.¹⁸ Increased CYP3A4 activity in young children is also supported by clinical investigations of cyclosporine that have demonstrated pharmacokinetic differences of a magnitude sufficient to impact both dosing regimen and drug efficacy.¹⁹ In contrast to other cytochromes P450, such as CYP1A2 and CYP2C9, neither sex nor menstrual cycle appears to alter the activity of CYP3A4, as assessed with either hepatic microsomal samples²⁰ or the *in vivo* pharmacologic probe midazolam.²¹

With respect to the impact of ontogeny on CYP3A4 activity, the most dramatic differences appear to occur during the first 6 months of life. As recently demonstrated by Lacroix et al,²² in an *in vitro* study that used human liver microsomes that were obtained from fetuses, neonates, infants, and children, CYP3A4 expression is transcriptionally activated during the first week of life and is accompanied by a simultaneous decrease of CYP3A7 expression. Additionally, these investigators demonstrated that CYP3A4 activity was extremely low in the fetus and attained 30% to 40% of adult activity at 1 month of postnatal age. Finally, this investigation demonstrated that adult levels of CYP3A4 were attained sometime between 3 and 12 months of postnatal age.

Pharmacokinetic evidence for this pattern of CYP3A4 ontogeny is reflected by studies with midazolam, a phar-

macologic probe that enables the assessment of both hepatic and intestinal CYP3A4/5 activity, depending on the route of administration (intravenous = hepatic; oral = hepatic + intestinal).²³ As demonstrated by Burtin et al,²⁴ the uncorrected plasma clearance of midazolam (liters per hour) at birth directly correlated with birth weight, a surrogate measure for CYP3A4 competence (Fig 4). These data suggest that CYP3A activity increases approximately 5-fold over the first 3 months of life, corroborating the *in vitro* findings of Lacroix et al.²² Finally, pharmacokinetic studies of the CYP3A4 substrates nifedipine, lidocaine, cyclosporine, and tacrolimus illustrate the profound and clinically important impact of ontogeny on CYP3A4 activity.¹⁶

In addition to CYP1A2 and CYP3A4, pharmacokinetic evidence supports a developmental dependence in the activity of CYP2C9. Biotransformation of phenytoin to the (S)-5-(4-hydroxyphenyl)-5-phenylhydantoin (S-HPPH) by CYP2C9 and the subsequent conjugation with glucuronic acid represents the principal metabolic pathway by which the drug is eliminated from the body. Under normal conditions, 95% of the HPPH recovered in the urine is the CYP2C9 product S-HPPH. Changes in phenytoin pharmacokinetics during development provide some insight into the maturation of CYP2C9. Specifically, phenytoin pharmacokinetic data reported by Chiba et al²⁵ demonstrated an age dependence in maximum velocity, which declined from approximately 14.0 mg/kg/d at 6 months of age to 8.0 mg/kg/d at 16 years of age. These changes were not associated with age-associated differences in the urinary excretion of HPPH. In addition, recent

TABLE III. Developmental patterns for the ontogeny of important drug-metabolizing enzymes in humans

Enzyme	Known developmental pattern
Phase I enzymes	
CYP2D6	Low to absent in fetal liver but present at 1 week of age; activity (ie, 20% of adult) by 1 month; adult competence by 3-5 years of age.
CYP2C9	Apparently absent in fetal liver; low activity in first 2-4 weeks of life, with adult activity reached by approximately 6 months; activity may exceed adult levels during childhood and decline to adult levels after conclusion of puberty.
CYP1A2	Not present in appreciable levels in human fetal liver; adult levels reached by approximately 4 months and exceeded in children at 1-2 years of age; adult activity reached after puberty.
CYP3A7	Fetal form of CYP3A that is functionally active (and inducible) during gestation; virtually disappears by 1-4 weeks of postnatal when CYP3A4 activity predominates, but remains present in approximately 5% of individuals.
CYP3A4	Extremely low activity at birth, reaching approximately 30%-40% of adult activity by 1 month and full adult activity by 6 months; may exceed adult activity between 1-4 years of age, decreasing to adult levels after puberty.
Phase II enzymes	
NAT2	Some fetal activity by 16 weeks' gestation; poor activity between birth and 2 months of age; adult phenotype distribution reached by 4-6 months, with adult activity reached by 1-3 years.
TPMT	Fetal levels approximately 30% of adult values; in newborns, activity is approximately 50% higher than adults with phenotype distribution, which approximates adults; exception is Korean children, where adult activity is seen by 7-9 years of age.
UGT	Ontogeny is isoform specific; in general, adult activity is reached by 6-24 months of age.
ST	Ontogeny is isoform specific and appears more rapid than that for UGT; activity for some isoforms may exceed adult levels during infancy and early childhood.

NAT2, N-acetyltransferase-2; TPMT, thiopurine methyltransferase; ST, sulfotransferase.

Adapted from Leeder JS, Kearns GL. Pharmacogenetics in pediatrics: implications for practice. *Pediatr Clin North Am* 1997;44:55-77. With permission.

pharmacokinetic data for the CYP2C9 substrate ibuprofen, from patients with cystic fibrosis who ranged in age from 5.5 to 29.6 years, demonstrated an inverse linear correlation between age and the apparent oral clearance for the drug.²⁶ Collectively, the data for phenytoin and ibuprofen support increased CYP2C9 activity in young children as compared with adolescents and young adults.

PHASE II PATHWAYS

In addition to the cytochromes P450, apparent age dependence exists for several phase II enzymes that are of quantitative importance for drug biotransformation.¹⁵ Studies of N-acetyltransferase 2 activity that used caffeine as a pharmacologic probe demonstrated the attainment of adult activity by approximately 4 to 6 months of postnatal age.²⁷ In contrast, the activity of thiopurine methyltransferase in newborn infants is approximately 50% higher than that observed in adults, with no discernable correlation with age, as demonstrated in a group of 309 Korean children between 7 and 9 years of age.²⁸ Pharmacokinetic studies of drugs that are substrates for various sulfotransferase isoforms (eg, acetaminophen) suggest that activity of this enzyme during infancy and early childhood exceeds levels in adults.¹⁵

Finally, as recently reviewed by de Wildt et al,²⁹ ontogeny appears to have a profound effect on the activity of several isoforms of uridine-5'-diphosphate-glucuronosyltransferases (UGTs). Studies that examined the impact of age on the disposition of several UGT substrates (eg, morphine, acetaminophen, ethinylestradiol, zidovudine, propofol, lorazepam, naloxone, diclofenac, bilirubin, and chloramphenicol) suggest that isoform-specific, age-related differences in UGT activity occur.

For example, pharmacokinetic investigations of selected substrates for UGT2B7 (eg, lorazepam, morphine, naloxone, and chloramphenicol) support marked reduction in the activity for this isoform (approximately 10% to 20% of adult levels) around birth with the attainment of adult competence between 2 months and 3 years of age.²⁹ For drugs such as morphine in which UGT2B7 catalyzes the biotransformation of the drug to an active metabolite (eg, morphine 6-glucuronide), delayed acquisition of morphine glucuronidation may have pharmacodynamic ramifications in the newborn as well. Despite these examples, current data for the UGTs do not permit the construction of a developmental profile for these enzymes as is apparent for certain CYPs (eg, CYP1A2 and CYP3A4). An information gap currently exists regarding the developmental and genetic aspects (ie, the possible role of polymorphisms) of UGT regulation and its potential impact on pediatric drug therapy.

Clearly, for some drug-metabolizing enzymes, there appears to be a definable profile by which ontogeny influences activity. Profiles for enzymes of major importance are summarized in Table III, which illustrates the importance of developmental regulation on the activity of both phase I and phase II drug-metabolizing enzymes.

DEVELOPMENTAL REGULATION

Despite emerging information on isoform-specific developmental differences in the activity of several important drug-metabolizing enzymes, little to no evidence clearly describes the regulatory events that are responsible for producing these differences at a cellular or molecular level. Although it was commonly believed that age-dependent differences in hepatic size (relative to total

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FIG 5. The disposition of another CYP2C9 substrate, phenytoin, has been shown to vary considerably based on the specific CYP2C9 genotype, with the presence of certain alleles (eg, CYP2C9*3) having an apparent dramatic influence on the dose versus plasma concentration relationship and, potentially, the therapeutic index for this drug. (Adapted from Mamiya K, Ieiri K, Shimamoto J, Yukawa E, Imai J, Ninomiya H, et al. The effects of genetic polymorphisms of CYP2C9 and CYP2C19 on phenytoin metabolism in Japanese adult patients with epilepsy: studies in stereoselective hydroxylation and population pharmacokinetics. *Epilepsia* 1998;39:1317-23. With permission.)

body size) in children was in part responsible for the apparent increased activity of many drug-metabolizing enzymes during childhood, Murry et al³⁰ demonstrated that liver volume in children is not associated with changes in the normalized (to weight and/or body surface area) plasma clearance of lorazepam, antipyrine, or indocyanine green. In a recent study, Relling et al³¹ examined the catalytic activities for selected pharmacologic substrates (ethoxyresorufin for CYP1A2, ethoxycoumarin for CYP2E1, midazolam or teniposide for CYP3A4/5, tolbutamide for CYP2C9, and 17 α -hydroxylation of paclitaxel for CYP2C8) using hepatic microsomes obtained from 13 children (0.5 to 9.0 years of age) and 18 adults (13 to 52 years of age). With the exception of slightly lower CYP2C9 activity found for children as compared with adults, there were no significant age-related differences noted for the remainder of the CYPs when catalytic activity was examined as a maximal rate per milligram of microsomal protein. Thus, apparent increases in the activity for selected cytochrome P450s reflected by pharmacokinetic studies of certain "substrates" do not appear to be supported by these in vitro findings.

Finally, it is possible that neuroendocrine determinants of growth and maturation may, in part, be responsible for observed developmental differences in the activity of certain drug-metabolizing enzymes. As recently postulated by Leeder and Kearns,¹⁵ the biologic effects of human growth hormone expressed during development may account for observed differences in the activity of specific drug-metabolizing enzymes. Support for this assertion was drawn from evidence that the human growth hormone can modulate the effect of many general transcrip-

tion factors (the demonstrated regulatory role for growth hormone in the expression of CYP2A2 and CYP3A2 in rats, the documented effects of human growth hormone treatment on the alteration of the pharmacokinetics for pharmacologic substrates of selected CYPs, and evidence of altered CYP1A2 activity, which appears to correlate with the pubertal height spurt).¹³

GENETIC POLYMORPHISM: IMPACT ON PHARMACOKINETICS AND PHARMACODYNAMICS

Underlying development as a determinant of either drug disposition or action in pediatric patients is the influence of pharmacogenetics. As recently reviewed,¹⁵ several important drug-metabolizing enzymes that, to some degree, demonstrate a dependence on development for their activity, are polymorphically expressed in humans. The therapeutic implications of genetic polymorphisms are illustrated by the following examples.

CYP2C9 is polymorphically expressed with point mutations, giving rise to 3 allelic variants (CYP2C9*1, 2, and 3). Inheritance of the CYP2C9*2 and/or CYP2C9*3 allele convey reduced enzyme activity as reflected by a 5.5- and-27 fold reduction, respectively, in catalytic activity towards S-warfarin, a CYP2C9 substrate.³² Kearns et al²⁶ have recently demonstrated that the plasma clearance of ibuprofen, a CYP2C9 substrate, in patients with cystic fibrosis shows a relationship to age that is not linear and is in part influenced by the CYP2C9 polymorphism. Additionally, the disposition of another CYP2C9 substrate (phenytoin) has been shown to vary considerably, based on the specific

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FIG 6. Concentration and AUC results, when fixed doses of a drug are administered to a pediatric study population, are depicted with the use of recent data for loracarbef. (Adapted from Abdel-Rahman SM, Blowey DL, Kauffman RE, Kearns GL. Comparative bioavailability of loracarbef chewable tablet vs oral suspension in children. *Pediatr Infect Dis J* 1998;17:1171-3. With permission.)

CYP2C9 genotype, with the presence of certain alleles (eg, CYP2C9*3) that have an apparent dramatic influence on the dose versus plasma concentration relationship (Fig 5) and, potentially, the therapeutic index for this drug.³³

Variations in the metabolism of the analgesic agent tramadol underscore the clinical importance of the genetic polymorphism for CYP2D6. Comprising only 6% of all hepatic CYPs, CYP2D6 is, nevertheless, an important enzyme because it catalyzes the biotransformation of over 40 therapeutic drugs, including many antihypertensive and psychopharmacologic agents.¹⁵ Tramadol biotransformation is catalyzed by CYP2D6 to *O*-Desmethyl-tramadol, a metabolite that is 6-fold more potent than the parent compound.³⁴ Phenotypic extensive metabolizers for CYP2D6 (approximately 93% of white patients and black patients) have been shown to experience a considerably stronger analgesic effect and a greater adverse effect profile from tramadol as the result of the formation of the potent *O*-Desmethyl-tramadol metabolite.³⁴ As expected, the plasma clearance of tramadol is also substantially reduced in individuals who are phenotypic CYP2D6 poor metabolizers.³⁴ Although preliminary data indicate that there is a complete concordance between CYP2D6 genotype and phenotype by 2 weeks of postnatal age,³⁵ it is not known whether the activity of this particular isoenzyme changes in an age-dependent fashion over the first 2 to 3 years of life.

Pharmacodynamic effects

In addition to having a dramatic impact on the pharmacokinetics of a drug, development can also directly influence pharmacodynamics. For example, a single intra-

venous dose of famotidine in neonates produced a sustained increase in gastric pH over a 24-hour period,⁸ a finding not seen in older infants and children in whom gastric pH returned to baseline (predose) levels at approximately 8 to 12 hours after a single intravenous dose.⁷ In the case of neonates, the sustained response to famotidine was attributed directly to the impaired clearance of the drug, which occurred consequent to developmental immaturity in glomerular filtration. Thus, an apparent age-dependent difference in the pharmacodynamics of famotidine appeared to have a pharmacokinetic basis. In contrast, Marshall and Kearns³⁶ recently demonstrated developmental differences in the pharmacodynamics of cyclosporine. In this study, the IC₅₀ for IL-2 expression, observed in peripheral blood monocytes obtained from infants younger than 12 months of age and exposed in vitro to cyclosporine, was approximately 50% of the value observed for older children. In this particular example, the pharmacodynamic differences appeared not to be the consequence of developmental dependence in pharmacokinetics but rather of the true drug-receptor interaction.

USE OF DEVELOPMENTAL PHARMACOLOGIC DATA IN STUDY DESIGN

Clearly, a unique set of issues are related to pediatric dosing and protocol development. The developmental changes that occur during childhood require an assessment of rapidly shifting drug disposition. These multiple developmental changes, however, affect 1 common and critical variable—drug exposure, which underlies drug efficacy, safety, and toxicity. The evaluation of drug

exposure (usually measured by determining the AUC after single or multiple drug doses) is a key step in the determination of the appropriate drug dose in children. For a given pediatric population, the AUC can be easily determined from an appropriate pediatric study cohort (eg, age-stratified, specific disease state) with the use of either classic (eg, multiple repeated blood samples for quantitation of parent drug or metabolite) or population-based pharmacokinetic approaches (eg, nonlinear mixed-effects modeling [NONMEM]). When linearity in the dose versus concentration relationship is proved, dose-normalized pediatric AUC values for a given drug are then simply compared with those from adult studies in which dose versus concentration (exposure) versus effect profiles are known. Concentration- and AUC-ranging results, when fixed doses of a drug are given to a pediatric study population, are illustrated in Fig 6, with the use of recent data for loracarbef.³⁷ In these instances, the need to repeat classic dose-ranging studies (such as those performed during phase I studies in adults) can be obviated. Finally, when AUC data are used to determine age-appropriate drug doses, it must be recognized that the "assumptions" that underlie this technique are predicated on a priori knowledge of "target" drug exposure levels and the assumption that the disease state or drug-receptor interaction between pediatric and adult patients is sufficiently similar, that is, comparable.

To standardize drug exposure in children, pediatric study protocol designs should take into consideration the known developmental changes in pediatric pharmacokinetics and pharmacodynamics. To accomplish this, several steps should be considered in the development of any pediatric protocol in which some component of a given drug's clinical pharmacology is to be evaluated:

1. Consider the impact of ontogeny on the activity of drug-metabolizing enzymes, especially the influence of known or expected developmental breakpoints that can significantly influence drug disposition, which in turn can affect the dose versus concentration (ie, exposure) versus effect relationship. Evaluate not only pharmacokinetic changes but also pharmacodynamic changes as a function of maturation.
2. Evaluate the potential influence of genetic polymorphism as regarding the activity of enzymes that are quantitatively important for drug biotransformation and the potential for genetic differences in drug response. Such data can be critical to the understanding of how genetic differences contribute to the variability between patients with regard to drug disposition and action (ie, response).
3. When a pharmacogenetic evaluation is deemed to be a useful component of a given clinical trial, examine both genotype and phenotype for relevant drug-metabolizing enzymes when possible. Although not expected consequent to the usual rigorous inclusion and exclusion criteria for a given study, discordance between genotype and phenotype can occur when concomitant treatments or dietary alterations are present in a given patient.

4. When studying drugs in patients with a particular medical condition, consider the impact of the disease state or its treatment on drug pharmacokinetics and pharmacodynamics. This is especially true for concomitant conditions (eg, sepsis or severe inflammation) in which the activity of drug-metabolizing enzymes might be altered by endogenous compounds (proinflammatory cytokines).
5. Select dynamic, sensitive, and specific surrogate markers for pharmacokinetic and pharmacodynamic assessments.

Taking these steps into consideration will facilitate the development of pediatric study protocols and the identification of age-appropriate dosing regimens.

CONCLUSIONS

Clearly, our current knowledge regarding the impact of ontogeny on drug disposition and action obviates the need to either "back into" pediatric dose selection by using subtherapeutic doses of drugs in an age deescalation paradigm (ie, administration to young adults first, then to adolescents, children, toddlers, infants, and neonates in succession) or to routinely repeat the well-established and orderly approach taken in the performance of phase I studies in adults. In many instances, available data can be used to design targeted pediatric trials capable of clearly defining age-appropriate drug dose (and exposure), provided that a priori estimates of pharmacokinetic parameters, the disposition profile, and the relationship between drug dose/exposure and both safety and efficacy in adults are known. Thus, the exploratory science of pediatric clinical trials must be hypothesis driven, not simply to confirm adult findings, but rather to define age-appropriate drug dosing regimens that are needed to assess critically the safety and efficacy of drugs that will be useful for the treatment of infants, children, and adolescents.

The author thanks Dr J. Steven Leeder for editorial assistance.

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