AGE SPECIFIC VARIATIONS IN DRUG METABOLIZING ENZYMES - IMPACT ON DRUG SAFETY EVALUATION IN CHILDREN

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Summary

The mechanisms of adverse drug reactions in children have been often related to the differences in patient response on pharmacokinetic level. Age-dependent variability in drug metabolizing enzyme systems (DMES) contributes to the wide range of drug responses observed in children. This review provides background information on a variety of DMES in biotransformation phase I and phase II that can vary as a function of developmental stage. The ontology of metabolizing systems is described via reference to pediatric studies involving therapeutic drugs and evidence from *in vitro* enzyme studies. The finding that drug response can be influenced by developmental profile of DMES has offered great hope for realizing patient-specific targeted therapy and approach to decrease adverse drug reactions. Prevention requires clinical monitoring in pediatric population.

Key words: *drug metabolizing enzyme, pediatric patients, adverse drug reactions*

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Introduction

The safety and tolerability of many pharmacologic agents in neonates, infants, children, and adolescents are not well established. Often the pharmacologic actions of drugs in children are not similar to those identified for adults; therefore, information obtained from research with adults cannot be applied directly.

Adverse drug reactions (ADR) related to drug biotransformation are common. Concentration-dependent toxicities resulting from drug accumulation due to immature drug metabolizing enzyme systems can be expected to occur in children with potentially life-threatening consequences [1]. The potential for concentration-dependent ADRs to occur as a consequence of delayed maturation of a specific drug biotransformation pathway is unique to newborns. There have been numerous examples of greater or unpredictable vulnerability of children to adverse effects. Verapamil was given to infants to convert supraventricular tachycardia on the basis of experiences with adults. After a series of infant deaths associated with its use, the different response in infants was recognized and verapamil is no longer used for this indication in infants. Desflurane, an inhalation anesthetic, provides rapid, smooth, and safe anesthesia induction in adult patients and appeared to be an ideal anesthetic for children. However, in pediatric patients it causes an unacceptable incidence of laryngospasm, breath holding, and hypersecretion when used as an induction agent. This life-threatening adverse event could not have been predicted from the results of trials with adults [2].

Another example is a syndrome of irritability, tachypnea, tremors, increased muscle tone, and temperature instability in neonates born to mothers receiving selective serotonin reuptake inhibitors (SSRIs) during pregnancy. Controversy exists as to whether these symptoms reflect a neonatal withdrawal (hyposerotonergic) state or whether they represent manifestations of serotonin toxicity analogous to the hyperserotonergic state attributed to SSRl-induced serotonin syndrome in adults [3-6]. Currently available data concerning the ontogeny of CYP2D6 and CYP3A4 in the first weeks of life are consistent with a hyperserotonergic state due to delayed clearance of paroxetine and fluoxetine (CYP2D6) or sertraline (CYP3A4) in neonates exposed to these compounds during pregnancy [7]. Furthermore, decreases in plasma SSRI concentrations and resolution of symptoms would be expected with increasing postnatal age and maturation of these pathways. Given that treatment of a withdrawal reaction may include administration of an SSRI, considerable potential exists for increased toxicity in affected neonates. As a result, resolution of the hyperserotonergic-hyposerotonergic pathogenesis is essential for appropriate management of SSRI-induced neonatal adaptation syndromes.

Theoretically, younger children may experience decreased efficacy or therapeutic failure with drugs such as codeine and tramadol that are dependent on functional CYP2D6 activity for conversion to the pharmacologically active species [8]. Infants and children appear capable of converting codeine to morphine achieving morphine: codeine ratios comparable to those of adults. However, in one study, morphine and its metabolites were not detected in 36% of children receiving codeine and codeine analgesia was found to be unreliable in the studied pediatric population and not related to the CYP2D6 phenotype. Ultrarapid CYP2D6 metabolism of codeine may result in opiate intoxication. CYP2D6 catalyzes the 0-demethylation of codeine to morphine. Ultrarapid metabolism of codeine results in high serum and breast milk concentrations of morphine and may have adverse effects in the breastfed neonate [7, 9, 10].

Considering the impact of ADRs on morbidity and mortality rates and the potential vulnerability of children to experience ADRs, bibliographic studies to evaluate the mechanisms and nature of ADRs in this population are warranted. Our goal is to outline the age dependent differences in drug metabolizing enzymes that need to be addressed if this area of study is to contribute to a children's drug safety. We also hope to provide some background and resources that can help shed light on these questions by showing where this information can fit into a framework for children's drug safety.

Animal pharmacokinetic information as a tool for evaluation of how development can affect drug response in children

Laboratory animals can be useful in a number of respects in providing information about DMES activity for possible children's risk for adverse drug reaction [11]. Diversity is one of the more daunting problems anticipated when studying children because of the numerous variability factors. Intersubject variability is substantially lower in homogeneous rodent populations provided by major animal suppliers. Uniform groups of animals of the same established genetic and husbandry backgrounds can be maintained under defined and carefully controlled conditions. Therefore, it is possible with animal studies to control more variables and to better focus on age-dependent differences in drug metabolism.

Comparative studies show that neonatal rodents are frequently more susceptible to xenobiotics, including drugs than adult animals, but such findings should be interpreted with caution when extrapolating to humans. Done (1964) and Goldenthal (1971) compiled the results of LD50 (median lethal dose) studies of several hundred xenobiotics in neonatal and mature rodents [12, 13]. The neonatal animals were more sensitive to many but not all the compounds. Almost all the age-dependent differences in LD50 values were less than an order of magnitude; indeed, most varied no more than 2- to 3-fold. More pronounced interage differences were seen for a few drugs, some of which (e.g., chloramphenicol, diazepam) are poorly metabolized and accumulate to toxic levels in human newborns. As full-term human newborns are more mature than their rodent counterparts with respect to liver metabolism, interage differences might be less pronounced in humans. However, maturation is much more rapid in rodents, such that even a few days of growth can result in marked disparity in chemical metabolism, disposition, and effects.

The maturation of hepatic drug metabolism in rats has been relatively well characterized [14, 15, 16]. Although rat liver is immature at birth with respect to many metabolic functions, certain cytochrome P450 (CYP) functions, epoxide hydrolase, and glucuronidation function reach adult levels within the first week to 10 days. Other functions such as glutathione transferase and aryl hydrocarbon hydroxylase take longer to develop [15]. Despite these known metabolic differences during development, findings of increased susceptibility in juvenile rodents have not been followed up to determine whether pharmacokinetic mechanisms underlie these susceptibility differences. Carbon tetrachloride, a chemical that also undergoes CYP2E1-catalyzed metabolic activation, was more hepatotoxic in 15-day-old than in 60-day-old male rats [17]. However, no accounts were located of animal studies relating susceptibility to injury by some CYP2E1 substrates to the time course of maturational changes in the chemical's metabolism. Thus, data from animal studies often can be useful in forecasting drug metabolism in humans when their limitations are considered. However, this conclusion is based on comparison of adult animals with human adults. The development of metabolic functions in early life is sufficiently different in rodents compared with humans to make direct extrapolation from juvenile animal studies difficult.

Human age specific variations in drug metabolizing enzymes
Prenatal development of drug metabolizing enzymes

As with most organ systems, the various drug-metabolizing systems undergo quantitative and qualitative changes during development. Table 1 summarizes data concerning some CYP isozymes and their occurrence in human fetal tissues [18-22]. During prenatal development, the activities of most enzymes that catalyze phases I and II reactions are lower than those in adults. As in the adult, the fetus exhibits substrate specificity in its ability to metabolize drugs, suggesting the existence of several sets of enzymes or isozymes, which may or may not be the same as those in the adult. These enzyme systems may be inhibited or induced by maternal pretreatment with a variety of drugs. Enzyme activity generally increases with gestational age. The ontogeny of each enzyme may be different, and the controlling mechanisms of maturation of enzyme activity are incompletely understood. Prenatal human and nonhuman primates exhibit higher levels of many metabolizing enzymes (especially P450s) than do commonly used laboratory species. As in the adult, the liver of the fetus appears to have the greatest capacity for chemical metabolism. The fetal adrenal, kidney, lung and brain also exhibit metabolic capabilities.

Table 1. CYP isozymes and their occurrence in human tissues

CYP	Adult liver	Adult brain	Fetal liver	*Fetal brain*
1A2	+++	–	–	
1B1	+	+		+
2A6	++		–	
2A7	+		–	
2B1/2B2	+	+		
2B6/2B7	+		–	
2C	+++		±	
2C8-19	+	+		+
2D6	+	+	±	
2E1	+	+	±	
2F1	–		–	
3A4	+++	+	±	
3A5	++		+	
3A7	+		+++	
4B1	–		–	

Legend: - not detected; + detected in small quantities; ++ available in moderate quantities; +++ available in high quantities; ± RNA detected, without protein confirmation.

Postnatal development profile of drug metabolizing enzymes

Pediatric patients have more complexity because fetuses and newborns may be phenotypically "slow" or "poor" metabolizers for certain drug-metabolising pathways acquiring a phenotype consistent with their genotype later in the developmental process as those pathways mature (glucuronidation, some CYP activities). It is apparent that not all infants acquire drug metabolism activity at the same rate due to the interaction between genetics and environmental factors. The CYPs, quantitatively the most important of the phase I enzymes, are heme-containing proteins that catalyze the metabolism of many lipophilic endogenous substances (steroids, fatty acids, fat-soluble vitamins, prostaglandins, leukotrienes, thromboxanes) and exogenous compounds, such as drugs. CYPs that have been identified as being important in human drug metabolism are predominantly found in the CYP1, CYP2, and CYP3 gene families.

Over the past several years children's pharmacokinetic databases have been developed in which therapeutic drugs tested in both children and adults are identified and key pharmacokinetic parameters are compared across ages [15, 23, 24]. The metabolism and clearance pathways of many of the drugs are known, making them useful indicators for particular pathways. For example, dextromethorphan and debrisoquine are known substrates for a particular cytochrome P450 (CYP) enzyme, CYP2D6; trimethadione, chlorzoxazone, and halothane are markers for CYP2E1 activity; morphine is predominantly processed by glucuronidation; and a host of antibiotics are not extensively metabolized but are mostly excreted unchanged by the kidneys [25- 28].

Table 2 shows data for some CYP and several phase II conjugation pathways. The data are compilation of information obtained from in vivo pharmacokinetic analyses of drugs with in vitro analyses of enzyme activity (EA) from liver samples. The combination of the two types of information for a given clearance pathway can provide an indication of how the pathway's function develops in the postnatal period. In vivo pharmacokinetic data have been analyzed across drugs that share a common mode of metabolic transformation in order to develop a more complete evaluation of the function of specific pathways. This data's compilation by pathway and age group indicates a fairly consistent pattern, that is, premature neonates, full-term neonates, and infants up to 6 months of age tend to have less metabolic and clearance capacity than adults. The exception in the chart is for enzyme expressed primarily in the fetal and early postnatal period: CYP3A7. This fetal form is replaced during the first year of life by corresponding (but not enzymatically equivalent) adult forms. Beyond 6 months, many CYP enzymes are sufficiently active that clearance in vivo is actually greater than that in adults. This appears to be due to the greater liver size and blood flow in children compared with adults [29]. Table 2 provides further evidence for this phenomenon in that the in vivo data indicate greater clearance capacity than suggested by the in vitro protein levels or enzyme activities for CYP1A2, CYP2E1, CYP2C9/19, CYP2D6, and CYP3A4, particularly at 6 months of age. Pathways function is shown as % adult.

Table 2. Postnatal development of metabolic enzyme function

Metabolic pathway	Substrates	Premature neonates	Full-term neonates	1 week–2 months	2–6 months	6 months–1 year	1–2 years	> 2 years
Phase I DMESs								
CYP1A1	CYP1A1 protein or enzyme activity was not detectable in microsomes from liver bank samples at any age, indicating very low constitutive levels							
CYP1A2	*Methoxyresorufin [30]* *Caffeine, Theophylline [23]*		2% Protein level 2% in vitro enzyme activity (EA) 11% in vivo t1/2	4 % Protein level 3% in vitro EA 23% in vivo t1/2	14 % Protein level 9% in vitro EA 81% in vivo t1/2	25 % Protein level 15% in vitro EA 175% in vivo t1/2	175% in vivo t1/2	54 % Protein level 35% in vitro EA 185% in vivo t1/2
CYP2E1	*Clorzoxazone [31]*		13% Protein level 27% in vitro EA	22% Protein level 39% in vitro EA	30% Protein level 47% in vitro EA	36 % Protein level 41% in vitro EA		82 % Protein level 83% in vitro EA
CYP 2C9/19	*Diazepam [9]* *Tolbutamide [23]*	21 % Protein level 33 % in vitro EA	30% in vivo	29 % Protein level 30 % in vitro EA	38 % Protein level 45 % in vitro EA	36 % Protein level 83% in vitro EA 182% in	36 % Protein level 83% in vitro EA 182% in vivo	130% in vivo

			t1/2			vivo t1/2	t1/2	t1/2
CYP2D6		13% Protein level	22% Protein level	34% Protein level	45% Protein level		88% Protein level	13% Protein level
CYP3A4	*Testosterone [32]* *alfentanil, carbamazepine, fentanyl, lidocaine, midazolam, nifedipine, quinidine, triazolam [23]*	 19% in vivo t1/2	17 % in vitro EA 50% in vivo t1/2	29 % in vitro EA 55% in vivo t1/2	37 % in vitro EA	46 % in vitro EA 200% in vivo t1/2	110 % in vitro EA 200% in vivo t1/2	 189% in vivo t1/2
CYP3A7	*dehydroepiandrosterone [32]*		1100 % in vitro EA	600 % in vitro EA	300 % in vitro EA	200 % in vitro EA		
Phase II DMESs								
Glucuronidation	*lorazepam, morphine, oxasepam, trichloroethanol, valproic acid, zidovudine [23]*	23 % in vivo t1/2	34 % in vivo t1/2	47 % in vivo t1/2	102 % in vivo t1/2	84 % in vivo t1/2	84 % in vivo t1/2	74 % in vivo t1/2
Sulfation/ glucuronidation	*acetaminophen [23] (Ginsberg et al, 2002)*		84% clearance		84% clearance		84% clearance	
Acetylation	**ratio of acetylated to nonacetylated metabolite of caffeine in urine [33]* ***sulfadimidine [34]*		*83% slow phenotype	** 12% in vivo caffeine N-acetylation	** 65% in vivo caffeine N-acetylation			*48% slow phenotype

CYP2D6 is involved in the biotransformation of >40 drugs, including beta-receptor antagonists, antiarrithmics, antidepressants, antipsychotics and morphine derivatives. Selective serotonin reuptake inhibitors (SSRIs), codeine and dextromethorphan are commonly used in pediatrics [35]. Very limited CYP2D6 activity is present in fetal liver in vitro (< 1% of adult values), but CYP2D6 is detectable in all samples from newborns [36]. Both CYP2D6 protein and catalytic activity progressively increase over 1st 28 days of life to 20% of activity observed in adults. In vivo data derived from a longitudinal phenotyping study conducted over the 1st year of life using dextromethorphan as a probe compound suggest that the CYP2D6 phenotype is concordant with genotype by 2 week of age [36]. Dextromethorphan phenotyping data from older children suggest that CYP2D6 catalytic activity in children is comparable to that in adults by at least 10 year of age, and probably much earlier. There are insufficient data from pharmacokinetic studies to determine the age at which the clearance of CYP2D6 substrates is comparable to that observed in adults [37].

CYP2C9 is involved in the biotransformation of diclofenac, ibuprofen, piroxicam, losartan, irbesartan, tolbutamide, warfarin, phenytoin [38]. In vitro studies show a progressive increase in CYP2C9 expression from 1-2% of mature levels in the 1st trimester to approximately 30% at term. Considerable variability (~35-fold) in expression is apparent over the 1st 5 month of life, with approximately ½ of the samples exhibiting values equivalent to those observed in adults. One interpretation of these data is broad interindividual variability in the rate at which CYP2C9 expression is acquired after birth, and in general, the ontogeny of CYP2C9 activity in vivo, as inferred from pharmacokinetic studies of phenytoin in newborns, is consistent with the in vitro results [37, 39].

Although several clinically important drugs are substrates for CYP2C9, the effects of variation in the enzyme activity is are most profound for drugs with a narrow therapeutic index, such as phenytoin, warfarin, and tolbutamide.

CYP2C19 is involved in the biotransformation of omeprazole, lansoprazole, nelfinavir, diazepam. Cimetidine and fluvoxamine are inhibitors of CYP2C19 and rifampin is inducer. In vitro, CYP2C19 protein and catalytic activity can be detected at levels representing 12-15% of mature values by 8 week gestation and remain essentially unchanged throughout gestation and at birth. Over the 1st 5 month of postnatal age, CYP2C19 activity increases linearly. Adult levels are achieved by 10 year of age, although variability in expression is estimated to be approximately 21-fold between 5 month and 10 year of age. The major source of this variability likely is pharmacogenetic in nature [37, 40].

Despite the increases in CYP2C19 activity observed in vitro over the 1st 5 month of life, the results of an in vivo phenotyping study with omeprazole in Mexican children implied that 17% of infants younger than 4 month of age could be classified as poor metabolizers, whereas none were detected beyond that point. In contrast, 20% of children 3-9 month old were classified as ultrarapid metabolizers compared with 6% of infants 1-3 months of age. For omeprazole, pharmacokinetic parameters comparable to those observed in adults are achieved by 2 years of age. Because proton pump inhibitors are also widely used clinically in pediatrics, pharmacogenetic as well as developmental considerations should guide dosing strategies in children [37].

The CYP3A subfamily consists of 4 members in humans (CYPs 3A4, 3A5, 3A7, and 3A43) and is quantitatively the most important group of CYPs in terms of human hepatic drug biotransformation. These isoforms catalyze the oxidation of many different therapeutic entities, several of which are of potential importance to pediatric practice [41]. CYP3A subfamily is involved in the biotransformation of calcium channel blockers (diltiazem, felodipine,

nimodipine, nifedipine, nitrendipine, verapamil); immunosupressive drugs (cyclosporine, tacrolimus); steroids (budesonide, cortisol, 17-beta estradiol, progesterone, testosterone); macrolides (clarithromycin, erythromycin, troleandomycin); benzodiazepines (alprazolam, midazolam, triazolam); opioids (alfentanyl, fentanyl, sufentanyl); HMG-CoA reductase inhibitors (lovastatin, simvastatin, atorvastatin); HIV protease inhibitors (indinavir, ritonavir, sequinavir).

CYP3A7 is the predominant CYP isoform in fetal liver and can be detected in embryonic liver as early as 50-60 days' gestation [42]. CYP3A4, the major CYP3A isoform in adults, is essentially absent in fetal liver, but increases gradually throughout childhood. Over the 1st 6 month of life, CYP3A7 expression exceeds that of CYP3A4, although its catalytic activity toward most CYP3A substrates is rather limited compared with that of CYP3A4.

CYP3A4 is also abundantly expressed in intestine, where it contributes significantly to the 1st-pass metabolism of orally administered substrates, such as midazolam. CYP3A5 is polymorphically expressed and is present in approximately 25% of adult liver samples studied in vitro [43].

Phase II enzymes include arylamine N-acetyltransferases (NAT1, NAT2), glucuronosyl transferases (UGTs), epoxide hydrolase, glutathione S-transferases (GSTs), sulfotransferases (SULTs), and methyltransferases (catechol 0-methyltransferase, thiopurine S-methyltransferase, several N-methyltransferases). Like the CYPs, UGTs, SULTs, and GSTs are gene families with multiple individual isoforms, each having its own preferred substrates, mode of regulation, and tissue-specific pattern of expression [44].

The UGT gene superfamily catalyzes the conjugation of several drugs used clinically in pediatrics, including morphine, acetaminophen, nonsteroidal anti-inflammatory drugs, and benzodiazepines, with glucuronic acid. Another potentially important deficit in children is glucuronidation during the first 2 months of life [45]. Because of the relative deficiency of glucuronide and high bilirubin levels (an endogenous glucuronidation substrate), it is possible that there will be greater internal exposure early in life to xenobiotics (or their metabolites) that require glucuronide conjugation. This is the case with the antibacterial drug chloramphenicol, in which a relative lack of glucuronidation contributed to its accumulation and toxicity (anemia) in infants [46, 47]. In contrast, some xenobiotics can undergo conjugation with alternative cofactors, which allows a shift in metabolic profile if the primary pathway is compromised or immature. This occurs with acetaminophen, where sulfation predominates early in life until glucuronidation takes over [48].

One of the earliest discovered and most widely recognized genetic polymorphisms is the arylamine N-Acetyltransferase-NAT2 polymorphism. Approximately 50% of whites and African- Americans in North America are phenotypically slow metabolizers, placing a substantial number of individuals at increased risk for the development of adverse drug effects, such as sulfasalazineinduced hemolysis, hydrazine or arylamine-induced peripheral neuropathy, procainamide- or isoniazid-induced systemic lupus erythematosus, and Stevens-Johnson syndrome or toxic epidermal necrolysis associated with sulfonamide administration. In vivo, with the use of caffeine as a phenotyping probe, all infants 0-55 days of age appear to be phenotypically slow acetylators, whereas 50% and 62% of infants 122-224 and 225-342 days of age, respectively, can be characterized as fast acetylators. Several independent studies indicate that maturation of the NAT2 phenotype occurs during the first 4 yr of life. Phenotype-genotype discordance is likely to be most apparent in the first 2-4 month of life, and drugs that are highly dependent on NAT2 function for their elimination should be used with caution [45].

Thiopurine S-methyltransferase (TPMT) is a cytosolic enzyme that catalyses the S-methylation of aromatic and heterocyclic sulfur-containing compounds, such as 6-mercaptopurine (6MP), azathioprine, and 6-thioguanine, used in the treatment of acute lymphoblastic anemia (ALL), inflammatory bowel disease, and juvenile arthritis, and to prevent renal allograft rejection. The relatively few patients with low to absent TPMT activity are at increased risk for severe myelosuppression if treated with routine doses of thiopurines; thus, they require a 10-15-fold reduction in dose to minimize this risk. Furthermore, these patients may be at increased risk for relapse as a result of inadequate or absent treatment with thiopurines. Given the expanding use of 6MP and azathioprine in pediatrics to treat inflammatory bowel disease and juvenile arthritis and to prevent renal allograft rejection, TPMT deficiency is not a trivial matter. Introduction of the TPMT phenotype or genotype determination into pediatric practice will lead to safer, more efficacious treatment in pediatric patient groups [45].

These findings are consistent with other pediatric studies in which clearance in childhood is slower than in adults for a variety of drugs and age groups [15, 24, 49]. There are also cases in which clearance is more rapid, particularly when the pediatric group was at least several months of age [15]. This type of age-specific and pathway-specific information in children should prove useful in predicting how children (particularly neonates) may differ from adults in drug response. Simply knowing the function of particular pathways may not be sufficient to predict in vivo handling of a drug at a particular age. Numerous pharmacokinetic factors are involved in drug processing and clearance, including absorption, protein binding, metabolic enzyme activities, renal and liver function. Therefore, a more comprehensive analysis may be needed to integrate the various factors at work and predict drug fate in children. The information provided in Table 2, combined with basic physiologic information, may make drug metabolism modeling of children more feasible.

Problem Formulation: Children as a Problem Target Groups

Children are difficult to study for a number of reasons. They are highly diverse from fetal through adolescent stages and beyond. Generalizations and defaults are not possible for such a sweeping range of development. Even within a narrow age range, there can be considerable variability, given the rapid and variable rate of development in early life. Another equally daunting problem is that it is not ethically feasible to introduce xenobiotics, even at trace amounts, into infants and children. Thus, there is very limited data in this age group.

Pharmacokinetic differences between children and adults with respect to the metabolism of therapeutic drugs have been recognized for years. These differences have spawned numerous clinical studies for the purpose of better titrating drug dosage to a particular age or body size [23, 24, 50, 51]. Although progress in pharmacokinetics has removed some of the uncertainty in crossspecies extrapolations in drug safety studies for adults, these principles have yet to be applied in a systematic manner to the drug safety of children. This is an important need, given that children's pharmacokinetics differ from adults in a number of ways: smaller body size; different ratios of fat, muscle, and water; higher breathing and metabolic rates per body weight; and immaturity of clearance systems and enzymatic reactions [15, 23, 50, 52]. Another obvious difference is that children are more diverse, undergoing a developmental program of growth and maturation that continuously alters how drugs are processed and cleared. Thus, incorporating children's pharmacokinetics and particularly drug metabolic enzymes, into drug safety assessment is complicated by the need to consider many developmental stages, ranging from *in utero* to adolescence, and by the extensive variability that can occur within each age group.

Recent findings of age-dependent variations on drug metabolizing enzymes have increased awareness of the existence and the prevention of unexpected and undesirable drug responses.

One approach in drug safety evaluation in children is clarification the questions regarding problem target groups, data analysis, including drug-specific analysis, child-specific analysis and to choose analytic option to run analysis. The most important questions that should help to a better evaluation are:

• Which age groups, dose routes, and target organs need to be considered based upon available drug safety information?

• What special factors in children need to be considered?

• What drug-specific and age group–specific enzyme data are available?

• What evaluation options are available?

The next step is the development of drug safety assessment approach. Drug-specific data regarding the fate of the drug(s) being evaluated are combined with children's data relating to the developmental profile of DMES (from *in utero* periods through adolescence). This will help an understanding of how children of various ages are likely to metabolize the drugs(s). Obviously, the best case is to have metabolism and disposition data for the drug(s) in children. However, it could be not so easy to find such data. The main approach to consider for filling the missing datais to use the available data for the therapeutic agents for which DMES data in children exist.

Typically, animal data, and in some cases, adult human data also, might be available for characterizing the drug's metabolism. Even if the target drug(s) has not been evaluated in children, a drug that is similarly processed may have been tested. Information for known therapeutic agent can help delineate the maturation of key pathways and how the drug in question will be handled at certain developmental stages.

Conclusions

The major consequence of age-dependent variations in drug-metabolizing enzymes is concentration-dependent toxicity due to impaired drug clearance. Drug metabolism in childhood is slower than in adults for a variety of drugs and age groups. For most CYPs, genotype-phenotype relationships are influenced by development in that fetal expression is limited (with the exception of CYP3A7) and functional activity is acquired postnatally in isoform-specific patterns. Clearance of some compounds appears to be greater in children relative to adults, obscuring the correlation between genotype and phenotype in neonatal life through adolescence.

From the published in vivo studies two different patterns of drug metabolism can be identified: activity is low immediately after birth, increases, then peaks at the young/mature adult level and, finally, decreases in old age (drugs catalysed by CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A3/4) and activity increases rapidly after birth to reach a level equivalent to that in the young/mature adult, then gradually decreases and finally decreasing faster in old age (drugs catalysed by CYP2E1). Further study of the changes in phase I and phase II biotransformation enzymes with age is warranted to help prevent adverse reactions and to guide us in tailoring therapy better for the individual patient.

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