

Perspective in Pharmacology

The Ontogeny of Human Drug-Metabolizing Enzymes: Phase II Conjugation Enzymes and Regulatory Mechanisms

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ABSTRACT

Changes in phase II drug-metabolizing enzyme expression during development, as well as the balance between phase I and phase II enzymes, can significantly alter the pharmacokinetics for a given drug or toxicant. Although our knowledge is incomplete, many of the phase II enzymes are expressed early in development. There is evidence for glutathione S-transferase A1/A2 (GSTA1/A2), GSTM, and GSTP1 in fetal liver, lung and kidney, although tissue-specific patterns and changes with time are observed. *N*-Acetyltransferase 1 (NAT1) activity also has been reported throughout gestation in fetal liver, adrenal glands, lung, kidney, and intestine. Only postnatal changes in NAT1 expression were apparent. Nothing is known about human NAT2 developmental expression. Some UDP-glucuronosyltransferase and sulfotransferase isoforms also are detectable in fetal liver and other tissues by the first or second

trimester, and substantial changes in isoform expression patterns, as well as overall expression levels, are observed with increasing maturity. Finally, expression of both epoxide hydrolases 1 and 2 (EPHX1 and EPHX2) is observed in fetal liver, and for the former, increased expression with time has been documented. Less is known about ontogenic molecular control mechanisms. Limited data suggest that the hepatocyte nuclear factor and CCAAT/enhancer binding protein families are critical for fetal liver drug-metabolizing enzyme expression whereas D element binding protein and related factors may regulate postnatal hepatic expression. There is a paucity of data regarding mechanisms for the onset of extrahepatic fetal expression or specific mechanisms determining temporal switches, such as those observed within the CYP3A and flavin-containing monooxygenase families.

Taking advantage of electrophilic functional groups already present on the molecule, or ones introduced during phase I metabolism, the phase II drug-metabolizing enzymes (DMEs) are characterized by their ability to conjugate xenobiotics using small molecular weight, organic donor molecules such as glutathione, UDP-glucuronic acid, or acetyl coenzyme A. These reactions generally result in pharmacological inactivation or detoxification, although instances of bioactivation are known. Conjugation products also can be substrates for specific transport enzymes, thus facilitating elimination from the body. Historically, research on DMEs has placed more emphasis on those catalyzing phase I versus phase II reactions. This also has been true with respect to studies on DME developmental expression. Given the importance of the conjugation enzymes in drug and toxicant disposition and, in particular, how the balance between phase I and phase II enzymes can dramatically alter pharmacokinetics

and therefore therapeutic efficacy and/or xenobiotic toxicity, this area deserves increased attention.

Advances in our understanding of phase II enzyme expression during ontogeny have been hampered by many of the same problems discussed for the phase I enzymes (Hines and McCarver, 2002). These include the difficulty in obtaining human tissue samples, the inappropriateness of animal models in the absence of validating human data, the failure to appreciate the impact of pharmacogenetics, the use of non-specific metabolic and immunological probes, and finally, the failure to appreciate the dynamic changes in gene expression during ontogeny.

Our understanding of molecular mechanisms controlling both phase I and phase II DME expression during ontogeny is even more incomplete than our knowledge of overall expression patterns. Yet, one can speculate about potential regulatory mechanisms based on the role of specific factors in

ABBREVIATIONS: DME, drug-metabolizing enzyme; GST, glutathione S-transferase; NAT, *N*-acetyltransferase; UGT, UDP-glucuronosyltransferase; EPHX, epoxide hydrolase; SULT, sulfotransferase; HNF, hepatocyte nuclear factor; C/EBP, CCAAT/enhancer binding protein; DBE, D element binding protein.

the developmental expression of other proteins. This concise review is intended to summarize our current understanding of phase II DME developmental expression in the human (see Table 1 for overall summary), highlight areas needing further study, and also discuss molecular mechanisms likely responsible for the developmental and tissue-specific expression patterns of both phase I and phase II enzymes. It is complemented by the companion article on the ontogeny of phase I DME (Hines and McCarver, 2002).

Glutathione S-Transferase

The glutathione S-transferase (GST) family of soluble, dimeric enzymes (EC 2.5.1.18) catalyze the conjugation of glutathione with a wide variety of electrophiles, generally resulting in detoxification and facilitated elimination. Thirteen different human GST subunits have been identified belonging to five different classes: α (GSTA1 through GSTA4), μ (GSTM1 through GSTM5), π (GSTP1), θ (GSTT1 and GSTT2), and ζ (GSTZ1). Subunit members can dimerize with members of the same class but not with members of other classes. An excellent review of the different GST enzymes, including a discussion of nomenclature, pharmacogenetics, gene regulation, and their role in toxicology, was recently published (Hayes and Pulford, 1995). However, a review of human GST ontogeny was not included.

Many of the early studies on GST developmental expression relied on substrate probes (e.g., Strange et al., 1985), and as such, conclusions drawn regarding differential expression suffer from a lack of specificity. Nevertheless, these studies did demonstrate differences in tissue-specific GST expression as a function of development. With the advent of specific immunological probes, more sophisticated studies were possible that allowed differentiation of the enzymes by GST class. Thus, using radioimmunological and immunohistochemical assays, Strange et al. (1989) were able to detect hepatic GSTA1 (182.4 to 247.2 pmol/mg cytosol protein) and GSTA2 (14.2 to 31.2 pmol/mg cytosol protein) expression as early as 10-weeks gestation. GSTA1 and GSTA2 expression levels increased 1.5- to 4-fold, respectively, to adult levels within the first 1 to 2 years of life. GSTM also was detected but exhibited the lowest expression level in the fetal samples (1.3 to 2.4 pmol/mg microsomal protein). At birth, GSTM

expression increased approximately 5-fold to adult levels. In contrast, the highest level of GSTP1 expression was observed in the early fetal samples (10 to 22 weeks gestational age, 18.0 to 25.2 pmol/mg cytosol protein), with subsequent decreases in expression during the second and third trimesters. Although still present in the neonatal period (5.0 pmol/mg cytosol protein), hepatic GSTP1 was nondetectable in the adult.

Immunohistochemical and radioimmunological assays also were used to ascertain the tissue-specific development of kidney and lung GSTs. In fetal kidney tissue less than 20 weeks gestational age, Hiley et al. (1989) were able to demonstrate GSTA1/A2 immunoreactive protein in the developing collecting tubules and primitive Bowman's capsule, but not in primitive glomerulus and mesenchymal tissue. As the nephron elongated with development, positive staining for GSTA1/A2 was observed along the entire length. However, in fetal tissue greater than 35 weeks gestational age, as well as neonatal and adult kidney, GSTA1/A2 was only present in the proximal tubule. For GSTP1, the fetal kidney expression pattern at less than 35 weeks gestational age was similar to that observed for GSTA1/A2. In fetal tissue greater than 35 weeks of age, expression was restricted to collecting tubules and the distal loop of Henle. These data were corroborated by Beckett et al. (1990) who demonstrated expression of GSTA1 and GSTA2 in 10 to 42 week fetal kidney at approximately 1.0 pmol/mg cytosol protein with an increase to 4.1 and 8.6 pmol/mg cytosol protein within the first 2 years of life, respectively. In contrast, GSTM decreased from 7.7 to 3.1 pmol/mg cytosol protein between the fetal and postnatal samples whereas GSTP1 levels remained constant (1.5 pmol/mg cytosol protein).

Cossar et al. (1990) performed a similar study in the developing lung wherein GSTP1 represents the major GST isoenzyme. At less than 20 weeks gestational age, the epithelial cells of the future airway are ductular columnar cells that will differentiate into type I and type II pneumocytes between 20 and 24 weeks. The ductular columnar epithelium at 12 to 18 weeks was strongly positive for GSTP1 expression, which decreased with differentiation. By 24 to 27 weeks, the distal airway epithelium was largely negative for GSTP1 expression, whereas immunoreactive protein was still detectable in the epithelial cells of the proximal airway. GSTA1 and GSTA2 were detectable at low levels in these same cells. Beckett et al. (1990) again corroborated these findings using radioimmunological analysis to show that GSTP1 expression decreased from 14.1 to 3.8 pmol/mg cytosol protein between the fetus and postnatal infants at 2 weeks to 2 years of age. GSTM decreased slightly (3.4 to 1.2 pmol/mg cytosol protein), but GSTA1 and GSTA2 remained relatively constant (1.0 and 0.3 pmol/mg cytosol protein, respectively). Consistent with, but expanding these earlier studies, a recent report by van Lieshout et al. (1998) revealed widespread expression of GSTA and GSTP1 in a single 8 week fetus. Both enzymes were present in liver, gastrointestinal tract, adrenal, and brain tissues, whereas only GSTP1 was observed in pancreas, lung, and kidney.

Although the studies described above provided valuable information demonstrating unique developmental and tissue-specific expression patterns for several GST enzymes, they were limited by probe specificity. With our current knowledge regarding the GST family and its 13 members, the

TABLE 1
Ontogeny of human hepatic phase II DME

Gene	Prenatal Trimester			Neonate	1 Month to 1 Year	1 to 10 Years	Adult
	1	2	3				
<i>GSTA1/A2</i>	+	+	+	+	+	+	+
<i>GSTM</i>	+	+	+	+	+	+	+
<i>GSTP1</i>	+	+	+	+	-	-	-
<i>NAT2</i>	+	+	+	+	+	+	+
<i>UGT1A1</i>	-	-	-	+	+	+	+
<i>UGT1A3</i>	?	+	+	+	+	+	+
<i>UGT1A6</i>	-	-	-	+	+	+	+
<i>UGT2B7</i>	?	+	+	+	+	+	+
<i>UGT2B17</i>	?	+	+	+	+	+	+
<i>EPHX1</i>	+	+	+	+	+	+	+
<i>EPHX2</i>	?	+	+	+	+	+	+
<i>SULT1A1</i>	?	+	+	+	+	+	+
<i>SULT1A3</i>	?	+	+	+	+	+	+
<i>SULT2A1</i>	-	-	+	+	+	+	+

+, activity or protein detectable; -, activity or protein not detectable; ?, not determined.

development of more specific assays and their application to the ontogeny of this important class of DME would be worthwhile.

N-Acetyltransferase

Recognition of *N*-acetyltransferase (NAT) (EC 2.3.1.5) importance to drug disposition dates back to the discovery of its role in the metabolism of the antituberculosis drug, isoniazid, and other therapeutics with similar chemical structures. Investigations of the mechanism of isoniazid toxicity led to the description of the rapid and slow NAT polymorphism, now known to result from genetic variation at the NAT2 locus (Hein et al., 2000b). Several studies have shown associations between polymorphisms at both the NAT1 and NAT2 loci and disease susceptibility (Hein et al., 2000a). Despite this appreciation of NAT and its importance to drug and toxicant metabolism, few studies have appeared on its developmental expression. Using *p*-aminobenzoic acid as a substrate probe, Pacifici et al. (1986) examined NAT1 activity in fetal tissues between 11 and 25 weeks gestation. Comparable activity (0.7 to 1.9 nmol/min/mg protein) was observed in fetal liver, adrenal glands, kidneys, lungs, and intestine, although there was no apparent association between activity and gestational age. Modestly greater activity was observed in the adult liver and intestine, but not in other tissues. A more complete description of both NAT1 and NAT2 ontogeny is needed.

UDP-Glucuronosyltransferase

The limited knowledge available on the expression of the UDP-glucuronosyltransferases (UGT) (EC 2.4.1.17) during development was recently reviewed by de Wildt et al. (1999). Although there have been significant advances in our understanding of UGT regulation since this review, these have not contributed directly to our knowledge of UGT ontogeny. For completeness, the data on the UGT family of enzymes will be summarized; however, reference should be made to the review by de Wildt et al. (1999) and citations therein for more extensive information.

There are 16 different UGT human enzymes. Nine of these are encoded at the UGT1 locus that consists of different functional promoters and first exons, which are alternatively spliced with common exons 2 through 5. UGT1A1, the enzyme most active toward bilirubin, is absent from the fetal liver. Expression is triggered by processes associated with birth and activity reaches adult levels by 3 to 6 months postnatal age (Burchell et al., 1989). In contrast, UGT1A3 activity is present in the fetal and neonatal liver at levels 30% of those observed in the adult (Burchell et al., 1989). This observation would suggest a developmental expression pattern including a postneonatal increase in expression, but this remains to be elucidated. Of the remaining members of the UGT1A subfamily, it is known that UGT1A6, the principal catalyst of acetaminophen glucuronidation, also is absent in the fetus, increasing slightly in the neonate, but not reaching adult levels until sometime after 10 years of age (Alam et al., 1977; Rollins et al., 1979).

Our previous poor understanding of UGT ontogeny and its clinical consequences is illustrated by the adverse reactions to chloramphenicol therapy observed in neonates, commonly referred to as gray baby syndrome. Although the precise UGT

enzyme responsible for this activity remains to be identified, it appears to be a member of the UBT2B subfamily. Gray baby syndrome was caused by the delayed onset of this UGT2B enzyme and subsequent high serum and tissue drug levels and resulted in the discontinued use of this drug in neonates. In contrast to UGT1, members of the UGT2 family are each encoded by discrete genes. Due to its specificity for morphine as a substrate, it is known that UGT2B7 is expressed at 10 to 20% of adult levels in the 15 to 27 week fetal liver with no apparent changes with increasing gestational age (Pacifici et al., 1982). Similar to other members of the UGT family of enzymes, UGT2B7 expression increases at birth, reaching adult levels by 2 to 6 months of age (Choonara et al., 1989). Finally, UGT2B17 is important in the metabolism of androgenic steroids. In the fetal liver, UGT2B17 is only present at 3% of adult levels, increasing to 13% in the neonate (Leakey et al., 1987). The time course for further increases in expression to adult levels is not known. Nothing has been reported regarding the ontogeny of the other 11 members of the UGT family of enzymes known to be present in the human.

Epoxide Hydrolase

Oxidation by one or more of the phase I enzymes often results in the formation of reactive, xenobiotic epoxides. The epoxide hydrolases (EPHX) (EC 3.3.2.3) are important for the ultimate detoxification of these intermediates, catalyzing the formation of *trans*-dihydrodiol derivatives. Originally thought to be localized solely in the endoplasmic reticulum, subsequent studies demonstrated distinct microsomal (EPHX1) and cytosol (EPHX2) enzymes. Multiple reports have appeared describing EPHX1 catalytic activity in several developing tissues. In fetal liver, adrenal, kidney, and lung ranging from 16 to 25 weeks gestation, EPHX1 activity, as measured using the substrate benzo[*a*]pyrene-4,5-oxide, was reported as 150 pmol/min/mg protein in the liver and adrenal glands, but only 50 pmol/min/mg protein in kidney and lung (Pacifici et al., 1983b). This same group subsequently demonstrated an increase in hepatic EPHX1 catalytic activity between 10 and 25 weeks gestation, although sample-size and intersubject variability prevented the authors from suggesting anything other than a weak correlation between activity and gestational age (Pacifici and Rane, 1983). Employing an immunological approach in eight fetal livers ranging from 17 to 27 weeks gestation, Cresteil et al. (1985) demonstrated EPHX1-specific content between approximately 40 and 400 pmol/mg microsomal protein whereas in four adult liver samples, the specific content ranged from 750 to 1200 pmol/mg microsomal protein. In both fetal and adult tissues, activity correlated well with protein content. Although the above studies demonstrated the presence of EPHX1 in fetal tissues with significant changes between the fetus and adult, information regarding temporal changes remained sparse. More complete data were contributed by Omiecinski and colleagues (1994) who reported hepatic EPHX1 activity as early as 7.5 weeks gestation (30 pmol/min/mg S9 protein) with a linear increase in activity to 22 weeks gestation (290 pmol/min/mg S9 protein). The activity at 22 weeks was approximately half that observed in adult liver. Good correlation was observed between EPHX1 activity and protein levels, but not between activity

and mRNA, suggesting multiple regulatory mechanisms. In the lung, an EPHX1 activity of approximately 16 pmol/min/mg S9 protein was observed as early as 12 weeks gestation, but did not change with time. In fact, EPHX1 expression in the fetal lung approached that seen in the adult, although activity in the latter ranged from 2 to 65 pmol/min/mg S9 protein.

Although fewer studies have appeared on EPHX2, it also is expressed in the fetus. Using styrene oxide as a substrate, EPHX2 exhibited activities of 230 pmol/min/mg protein in the fetal liver (10 specimens at 15 to 24 weeks gestation) with an approximate 4-fold increase in the adult (14 specimens, 29 to 69 years of age) (Pacifci et al., 1983a). A subsequent study with a more sensitive assay for trans-stilbene oxide hydration demonstrated EPHX2 as early as 14 weeks in the fetal liver (Pacifci et al., 1988). Although considerable inter-individual variability was observed, no change in activity occurred up to 27 weeks, mean activity being 55.2 ± 89.6 pmol/min/mg cytosol protein. Similar to the earlier report, adult hepatic activity was 5-fold greater than that observed in the fetus. These data indicate a significant change in hepatic EPHX2 expression sometime between 27 weeks gestation and 30 years of age that warrants further investigation. EPHX2 activity comparable to that seen in the liver was demonstrated in the fetal kidney, adrenal glands, intestine, and lungs. However, developmental changes in extrahepatic EPHX2 expression were less apparent.

Sulfotransferase

The sulfotransferase (SULT) gene family encodes at least 11 distinct enzymes that catalyze the sulfate conjugation of a variety of endogenous and exogenous chemicals using 3'-phosphoadenosine-5'-phosphosulfate (PAPS) as a donor (Glatt, 2000) (R. B. Raftogianis, M. W. H. Coughtrie, I. D. Beckmann, R. R. Freimuth, J. Buck and R. M. Weinshilboum manuscript submitted). Although the presence of and changes in SULT activity have been well documented during human development, these studies were performed with substrate probes that may or may not be specific. An attempt will be made to assign these activities to a specific enzyme in this review, with obvious limitations. With the development of more specific probes, a re-examination of SULT ontogeny would be important.

A comparative study between the ontogeny of dopamine SULT (SULT1A3) and *p*-nitrophenol SULT (SULT1A1) (EC 2.8.2.1) was one of the first to demonstrate the expression of substantial SULT in fetal tissue and changes with development (Cappiello et al., 1991). These investigators demonstrated higher SULT1A3 activity in fetal liver and kidney (six tissue samples from individuals ranging in gestational age from 18 to 25 weeks) than in the same adult tissues (six adult samples, ranging in postnatal age from 22 to 76 years) but comparable activity in lung and intestine. In contrast, SULT1A1 activity was higher in all four adult tissues. Consistent with this study, Pacifci et al. (1993) examined SULT1A3 activity using the β_2 -adenoreceptor agonist, ritodrine, as a substrate in 48 fetal liver samples (14 to 27 weeks gestational age) and six tissue samples each from fetal lung, kidney, and intestine. Although highly variable, comparable activity was observed in liver, kidney, and lung at approximately 50% of the activity observed in the intestine. In the adult, SULT1A3 activity was reduced by

half in the liver and by approximately 90% in the kidney but was essentially unchanged in the intestine and lung. Despite the differences between fetal and adult liver, there was no apparent association between gestational age and SULT1A3 activity, suggesting the changes observed occurred late in pregnancy or postnatally. Also consistent with these data, Gilissen et al. (1994) demonstrated the presence of hepatic *N*-hydroxy-4-acetylamino-biphenyl, *N*-hydroxy-4-aminobiphenyl, and 1-naphthol SULT activity, most likely attributable to SULT1A1, as early as 15 weeks gestation. Of interest, these investigators failed to see any significant changes in activity during gestation or at 1 or 1.5 years postnatal age. Thus, if SULT1A1 activity does increase in the adult, this may not occur until quite late in childhood development, or perhaps early adult.

Barker et al. (1994) examined the ontogeny of SULT2A1 (EC 2.8.2.2) using both a specific rabbit anti-rat SULT2A1 antibody and dehydroepiandrosterone as a substrate probe. In the liver, both activity and protein levels were low to nondetectable before 25 weeks gestation, but then increased substantially during the latter half of gestation to approach adult levels in the neonate. Five-fold higher activity and specific content were observed in the adrenal glands, but no change was observed with fetal or postnatal development. A similar SULT2A1 developmental expression pattern was observed in the kidney, although activity in this tissue was 10% of that observed in the liver. Tissue-specific expression also was observed in the kidney, with immunoreactive protein observed in the proximal and distal tubules, loops of Henle, and collecting ducts but not the vascular glomerulus.

Less extensive studies suggest that SULT1E1 (estrogen sulfotransferase) (EC 2.8.2.4) is expressed in fetal lung, although it is not known how these expression levels compare with the adult (Jones et al., 1992). More recently, SULT1C1 mRNA was shown to be expressed in fetal kidney and to a lesser degree, fetal liver. Substantially higher expression was observed in adult stomach, thyroid gland, and kidney, suggesting changes with maturation (Her et al., 1997). No studies have appeared on the ontogeny of SULT1A2, SULT1B1, SULT1C2, SULT2B1, or SULT4A1.

Regulatory Mechanisms Controlling DME Expression during Ontogeny

What do we know about molecular mechanisms controlling specific DME developmental expression? Sadly, there have been few studies that directly address this question. One can speculate about the possible involvement of one or more transcription factors based on our functional knowledge of various enzyme promoters and our knowledge regarding the role of various regulatory proteins during ontogeny, although some of this information is derived from animal models and may or may not directly extrapolate to the human. Members of the hepatocyte nuclear factor (HNF) family of transcription factors are important for the regulation of several members of the *CYP2* gene family. Early studies by Ueno and Gonzalez (1990) demonstrated a role for HNF1 α in regulating rat *CYP2E1* basal expression whereas Chen et al. (1994) have shown that HNF4, an orphan nuclear receptor, also is important in regulating human *CYP2C* expression. Cairns et al. (1996) provided convincing evidence that HNF4 and the related factor, COUP-TF1 (chicken ovalbumin upstream pro-

Summary

Substantial changes in phase II DME expression occur during development that will have a profound impact on xenobiotic disposition and clinical outcome. Adding substantially to the complexity, the ultimate disposition of many drugs and toxicants depends not only on the spectrum of phase I and phase II enzymes but the dynamic balance between these two classes of DME. Yet, our understanding of these changes remains inadequate, and therefore, our ability to predict adverse reactions and develop effective therapies for the fetus, neonate, infant, and child remains compromised. Equally important will be studies to further our knowledge of genetic differences that underlie the large interindividual variation in DME expression and mechanisms responsible for controlling DME during ontogeny.

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