The Ontogeny of Human Drug-Metabolizing Enzymes: Phase I Oxidative Enzymes

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ABSTRACT

Although some patterns are beginning to emerge, our knowledge of human phase I drug-metabolizing enzyme developmental expression remains far from complete. Expression has been observed as early as organogenesis, but this appears restricted to a few enzymes. At least two of the enzyme families that are expressed in the fetal liver exhibit a temporal switch in the immediate perinatal period (e.g., CYP3A7 to CYP3A4/3A5 and FMO1 to FMO3), whereas others show a progressive change in isoform expression through gestation (e.g., the class I alcohol dehydrogenases). Many of the phase I drug-metabolizing enzyme exhibit dynamic perinatal expression changes that are regulated primarily by mechanisms linked to birth and secondarily to maturity. A few of these enzymes are not detectable until well after birth, suggesting that birth is necessary but not sufficient for the onset of expression (e.g., CYP1A2). Tissue-specific expression adds to the complexity during ontogeny. For example, CYP3A7 expression is restricted to the fetal liver. However, with few exceptions, complete temporal relationship information during development is not known. Furthermore, most studies have concentrated on hepatic expression and much less is known about extrahepatic developmental events.

It is well recognized that substantial changes in pharmacokinetics occur during human development. That these changes contribute to differences in therapeutic efficacy and toxicant susceptibility has been well documented. Metabolism is a major determinant of pharmacokinetics. Although changes in drug-metabolizing enzyme (DME) expression during development are well recognized, the knowledge needed to understand and predict therapeutic dosing and avoidance of toxicity during maturation is incomplete. Historically, DME activity has been divided into two categories, phase I and phase II. Depending on the chemical nature of a xenobiotic, the former is characterized by oxidative metabolism resulting in either: 1) pharmacological inactivation or activation, 2) facilitated elimination, and/or 3) addition of reactive groups for subsequent phase II conjugation. This concise review is intended to summarize our current understanding of phase I DME developmental expression in the human (see Table 1 for overall summary) and highlight areas needing further study. It is complemented by the companion article on the ontogeny of phase II DME and DME regulation during development (McCarver and Hines, 2002).

Advances in this field have been hampered by several problems. Foremost has been the ethical and logistical problems in obtaining tissue samples of suitable quality for in vitro studies, particularly for those examining RNA. The need for studies using human tissues became obvious with the realization that significant species differences exist in the DME structural genes and also in developmental control mechanisms. Furthermore, variation in human metabolic capacity, including variation during development, is well documented. Advances in pharmacogenetics have revealed specific molecular mechanisms accounting for interindividual variability, including both structural and regulatory polymorphisms that vary in frequency across ethnic groups. Regulatory polymorphisms that uniquely impact developmental expression also are likely. Thus, direct extrapolation of developmental expression data from animal models to the human is inappropriate without human data to validate such models. Another problem has been the historic lack of specific substrate and immunological probes. Because of this deficiency, the presence or absence of one or more members of a gene family may be inferred from past in vivo and in vitro

ABBREVIATIONS: DME, drug-metabolizing enzyme; EROD, ethoxyresorufin O-deethylase; RT-PCR, reverse transcriptase-coupled polymerase chain reaction amplification; Ah, aryl hydrocarbon; CAR, constitutive androstendione receptor; PXR, pregnane X receptor; ADH, alcohol dehydrogenase; CYP, cytochrome P450; FMO, flavin-containing monoxygenase.
metabolic and immunochemical studies, but not specific enzymes. A long-standing conundrum has been the apparent paradoxical relationship between historical in vivo metabolic data and in vitro enzyme levels. Weight-corrected drug clearance in pediatric patients generally is reported as higher than adult values, despite the almost universal observation of reduced enzyme levels in children. This may be partially explained by the allometric model discussed by Anderson et al. (1997), but other physiological differences between these populations also must contribute. Finally, the different stages of ontogeny are characterized by dynamic changes in gene expression. Thus, in vitro studies drawing conclusions based on a small number of tissue samples, representing a narrow time window must be viewed cautiously.

### Ontogeny of the Cytochromes P450

Total cytochrome P450 (EC 1.14.14.1) content in the fetal liver is 30 to 60% of adult values. At birth, increases are observed such that total hepatic cytochrome P450 content approaches adult levels during the first 10 years of life (Shimada et al., 1996b). However, significant differences in expression are observed among the three gene families important for xenobiotic metabolism, as well as among the 24 individual enzymes encoded by these families.

**CYP1 Gene Family.** The three members of the CYP1 gene family, CYP1A1, CYP1A2, and CYP1B1, are essential for the metabolic disposition of environmental polycyclic and halogenated aromatic hydrocarbon, aromatic amines, estradiol, and several other compounds (Parkinson, 1996). Evidence for the presence of constitutively expressed CYP1A1 enzyme in the fetus is compelling. In 1987, Pasanen et al. (1987) reported ethoxyresorufin O-deethylase (EROD) activity in seven fetal liver samples (unreported gestational age) that was inhibited to a variable extent by an anti-rat CYP1A1 monoclonal antibody. Similarly, Yang et al. (1995) demonstrated EROD activity (6.2 pmol/min/mg of microsomal protein) in 8 to 10 pooled embryonic liver samples at 7 to 9 weeks estimated gestational age. In the latter case, activity was inhibited by both a polyclonal rabbit anti-rat CYP1A1 antibody and 7,8-benzoflavone. CYP1A1 mRNA was also detectable by RT-PCR, providing convincing evidence for enzyme expression as early as liver organogenesis. Immunological evidence (Murray et al., 1992; Shimada et al., 1996b), as well as the expression of EROD activity (7.3 ± 5.5 pmol/min/mg of microsomal protein) and the metabolic activation of benzo[a]pyrene 7,8-diol and aflatoxin B1 (Shimada et al., 1996b) also provided strong evidence for CYP1A1 expression later in development, i.e., 11 to 20 weeks of gestation. These data are consistent with the report by Omiecinski et al. (1990) in which CYP1A1 mRNA was detectable in fetal liver, lung, and adrenal, but not kidney tissue between 6 and 12 weeks, 8 and 21 weeks, and 11 and 17 weeks gestational age, respectively. In each case, CYP1A1 mRNA expression declined with increasing age. In contrast, constitutive CYP1A1 expression is not generally detectable in adult tissues. Thus, the suppression of this activity must occur sometime late in prenatal, perinatal, or early childhood development.

CYP1B1 expression and its role in procarcinogen metabolism has been of considerable interest. Yet, only two studies have examined CYP1B1 expression during ontogeny. Hakkola et al. (1997) reported CYP1B1 mRNA expression in fetal liver (12 to 19 weeks gestation) only in these three of the six samples examined and with considerable variability among the three positive samples. In contrast, Shimada et al. (1996a) were unable to detect CYP1B1 mRNA in either fetal or adult liver. Extensive extrahepatic fetal CYP1B1 mRNA expression has been readily demonstrated, including fetal kidney, brain, adrenal gland, and lung (Hakkola et al., 1997).

Given the ability of CYP1B1 to activate estrogen and polycyclic aromatic hydrocarbons to genotoxic metabolites (Parkinson, 1996), the expression of CYP1B1 in steroidogenic and steroid-sensitive tissues (Muskhelishvili et al., 2001), the recognized role for steroids in development, and the putative link between mutations in this gene and congenital glaucoma (Bejiani et al., 1998), a more exhaustive study of CYP1B1 ontogenic expression is warranted.

In contrast to CYP1A1 and CYP1B1, CYP1A2 does not play a role in fetal xenobiotic metabolism. Using specific immunological probes and/or RT-PCR, several groups reported an inability to detect fetal liver CYP1A2 expression in specimens from individuals ranging in gestational age from 11 to 24 weeks (Mäenpää et al., 1993; Hakkola et al., 1994; Yang et al., 1995; Shimada et al., 1996b). Tateishi et al. (1997) dem-

### Table 1

**Ontogeny of human hepatic phase I DME**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Prenatal Trimester</th>
<th>Neonate</th>
<th>1 Month to 1 Year</th>
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*+, activity or protein detectable; −, no detectable activity or protein; ?, not determined; ±, activity or protein detectable, but in only a fraction of the samples examined; +?, presence or absence is controversial.*


shown CYP1A2 protein expression at 10% of adult levels in two individuals 4 weeks of age. A more recent corroborating study by Sonnier and Creasteil (1998) further demonstrated that CYP1A2 expression was absent during the fetal and neonatal periods, then increased in infants between 1 and 3 months of age. Even at 1 year, CYP1A2 levels were only 50% of those seen in the adult.

Thus, the three members of the CYP1 gene family, all regulated at least in part by the Ah receptor, display different hepatic developmental expression patterns. Although Ah receptor expression has been observed in fetal tissues and likely is involved in the expression of CYP1 genes after exposure of the fetus to certain environmental substances, the differential expression of these genes during development suggests the involvement of important regulatory mechanisms independent of the Ah receptor.

**CYP2 Gene Family.** CYP2 is the most diverse human cytochrome P450 gene family with eighteen members. Although not expressed at high levels in the adult liver, members of the CYP2A gene family are found at relatively high levels in several extrahepatic tissues, including the olfactory mucosa (Su et al., 1996). This observation, together with the active role of CYP2A in the metabolism of nicotine, tobacco smoke procarcinogens, and other inhaled toxicants (Li et al., 1996), has generated considerable interest. CYP2A6 and CYP2A13, but not CYP2A7, were readily detectable using both immunological probes and RT-PCR in seven of eight human fetal nasal mucosa samples from individuals ranging in age from 13 to 18 weeks. However, expression was 1 to 5% of adult levels. Furthermore, although the sample size was small, expression tended to be higher in the older samples, i.e., 15 to 18 weeks (Gu et al., 2000). Based on this trend and the observed high expression levels in adult nasal mucosa, one would speculate that CYP2A6/2A13 expression would continue to increase in the third trimester. However, such a time course has not been determined. None of the CYP2A enzymes appear to be expressed in fetal liver (Mäenpää et al., 1993; Hakkola et al., 1994; Shimada et al., 1996b; Gu et al., 2000). Yet, data from Tateishi et al. (1997) demonstrated expression of hepatic CYP2A6 at or near adult levels by 1 year of age. Additional studies clearly are needed to clarify the CYP2A hepatic and extrahepatic developmental expression pattern.

CYP2B6 has been strongly implicated in the metabolism of several important therapeutics and toxicants (Parkinson, 1996). Expression of CYP2B6, which is controlled in part by the nuclear orphan receptor CAR (Sueyoshi et al., 1999) and PXR (Goodwin et al., 2001), is generally detectable in adult liver. However, considerable interindividual variation is observed that is partly attributable to polymorphisms within the CYP2B6 structural gene (Lang et al., 2001). Using either an immunological probe (Mäenpää et al., 1993) or RT-PCR (Hakkola et al., 1994), CYP2B6 was not detectable in fetal liver at 11 to 24 weeks gestation. A single study suggests expression at approximately 10% of adult levels within the 1st year of life (Tateishi et al., 1997). Thus, similar to the CYP2A gene family, we know little about when or by what mechanism(s) CYP2B6 expression is activated during development.

There are four members of the human CYP2C gene family, CYP2C8, CYP2C9, CYP2C18 and CYP2C19. Of these, the enzymes encoded by CYP2C9 and CYP2C19 are the most abundant in adult human liver (Shimada et al., 1994). The CYP2C gene products are important for the metabolism of numerous therapeutics, including phenytoin, tolbutamide, diazepam, omeprazole, tienilic acid, and diclofenac (Parkinson, 1996). This gene family also is highly polymorphic, with well characterized, functional variants at the CYP2C9 and CYP2C19 loci. Early studies by several groups suggested low, but detectable CYP2C expression in the fetal liver (Mäenpää et al., 1993; Hakkola et al., 1994). However, a more recent study by Treluyer et al., (1997) failed to detect CYP2C protein or tolbutamide metabolism in 50 fetal livers ranging in age from 16 to 40 weeks. Interestingly, CYP2C8, CYP2C9, and CYP2C18 transcripts were detectable but at levels approximately 10% of the adult values, suggesting possible post-transcriptional control mechanisms. CYP2C enzyme expression appears to be activated by a mechanism associated with birth but independent of gestational age. Low but detectable enzyme levels are seen within the first 24 h after birth. Levels approximately 30% of the adult are seen in the neonate, which remain constant up to 1 year of age. The increase in CYP2C expression in the neonate appears largely due to CYP2C9. However, the time course for the subsequent increase in each CYP2C enzyme to adult levels has not been determined. The importance of variability in cytochrome P450 developmental changes is underscored by the report by Treluyer and colleagues (Treluyer et al., 2000) of a 3-fold increase in CYP2C9 expression that was associated with sudden infant death syndrome. The authors also showed a parallel increase in arachidonic acid oxidation to epoxyeicosatrienoic acid (EET) and dihydroxyeicosatrienoic acid (HETE), reactions known to be mediated by CYP2C8 and CYP2C9. Although unproven, this increase in EET and HETE may contribute to decreased vascular tone and be a sudden infant death syndrome risk factor.

Although CYP2D6 represents only 2% of total adult hepatic cytochrome P450 (Shimada et al., 1994), it mediates the metabolism of multiple therapeutics, including antitussives, antihypertensives, and tricyclic antidepressants (Parkinson, 1996). Based on the inability to detect the O-demethylation of dextromethorphan, early studies by Ladona et al. (1991) suggested the absence of CYP2D6 expression in fetal liver. With three 11- to 13-week fetal liver samples, Shimada et al. (1996b) also failed to detect CYP2D6 immunoreactive protein. In contrast, with a much larger sample size (60 fetal samples), Treluyer and colleagues (1991) reported both CYP2D6 protein and mRNA in fetal liver at less than 30 weeks gestational age, but in only 30% of the specimens examined and at only 5% of adult levels. In specimens of greater than 30 weeks gestational age, expression was detectable in about 50% of the samples, but still at only 15% of adult levels. A single report also has described the expression of CYP2D6 in human fetal brain (Gilham et al., 1997). The reported interindividual variability in expression is consistent with the known polymorphic expression in adults, but also may reflect a regulatory polymorphism(s) controlling developmental expression. Similar to CYP2C9, CYP2D6 protein increased significantly after birth, independent of gestational age, and continued upward to levels about 50 to 75% of adult values during the neonatal period. Interestingly, steady-state CYP2D6 mRNA only correlated with protein levels in the adult, not in the fetus, suggesting the possibility
of CYP2D6 post-transcriptional control mechanisms during ontogeny (Treluyer et al., 1991).

CYP2E1 is active in the metabolism of short-chain, dialkyl nitrosamines, organic solvents such as ethanol, carbon tetrachloride, chloroform, toluene, benzene, and trichloroethylene, and therapeutics such as acetaminophen, caffeine, chlorzoxazone, diphenhydramine, and several anesthetics. Through multiple, poorly characterized regulatory mechanisms, this enzyme is induced by many of its organic solvent substrates (Parkinson, 1996). Although some investigators have been unable to detect CYP2E1 expression in the fetal liver (Hakkola et al., 1994; Shimada et al., 1996b; Vieira et al., 1996), studies by Raucy and colleagues (1996) and Juchau and colleagues (1997) would argue otherwise. These groups reported detectable hepatic CYP2E1 at 10 to 30% of adult levels as early as 16 weeks gestation, remaining constant up to 24 weeks. Of particular interest to those studying alcohol and organic solvent teratogenesis, CYP2E1 also has been reported to be expressed in the developing human brain at levels greater than that seen in hepatic tissue and as early as 7 to 9 weeks gestation, i.e., during the period of organogenesis (Brzezinski et al., 1999). Vieira et al. (1996) demonstrated a rapid activation of CYP2E1 expression at birth. These changes are accompanied by a loss of CpG methylation in the 5' region of the gene, suggesting a role for DNA methylation in controlling CYP2E1 expression during ontogeny. Similar to what has been observed for other members of the CYP2 gene family, onset of postnatal expression also appears independent of gestational age. Hepatic CYP2E1 expression increases gradually, reaching 30 to 40% of adult levels by one year and essentially 100% of adult levels by 10 years (Vieira et al., 1996).

One of the most recently discovered cytochromes P450, CYP2J2 is expressed at significant levels in the adult human heart, gastrointestinal tract, kidney, liver, and lung (Wu et al., 1996). This enzyme catalyzes the oxidative inactivation of retinoic acid, as well as the epoxidation of arachidonic acid to several biologically active compounds (Scarborough et al., 1999). A recent report by Gu et al. (2000) demonstrated expression of CYP2J2 protein in 13 to 18 week fetal liver and olfactory mucosa at levels comparable to that observed in the adult. Given the biological activity of CYP2J2 substrates and/or products, particularly during development, and the unusually high expression levels of this enzyme during ontogeny, it will be important to further elucidate the CYP2J2 developmental expression pattern. Possible genetic variability at this locus and its impact on ontogeny also would be of considerable interest.

**CYP3 Gene Family.** The CYP3A4 and CYP3A5 gene products account for 30 to 40% of the total cytochrome P450 in the adult liver and small intestine where they participate in the metabolic disposition of a wide variety of therapeutics and toxicants. Large interindividual variability in CYP3A expression is observed that may be partially explained by regulation through PXR and CAR, and partially by genetic variability (Kuehl et al., 2001). Early studies by Wrighton et al. (1988) identified CYP3A7 as a member of the CYP3A subfamily uniquely expressed in fetal liver. Subsequent studies demonstrated the differential expression of this enzyme in the fetus with a switch to CYP3A4/3A5 in the adult (Schuetz et al., 1994; Yang et al., 1994). Fetal hepatic CYP3A7 is detectable as early as 50 to 60 days gestation with continued significant levels of expression through the perinatal period (Lacroix et al., 1997). Expression begins to decline after the first postnatal week, reaching nondetectable levels in most individuals by 1 year of age. Hepatic CYP3A4/3A5 expression begins to dramatically increase at about 1 week of age, reaching 30% of adult levels by 1 month (Lacroix et al., 1997). Because of the simultaneous decline in CYP3A7 and increase in CYP3A4/3A5, total CYP3A protein expression over the entire developmental period remains constant (Lacroix et al., 1997). However, because CYP3A7 and CYP3A4 do exhibit differences in substrate specificity and catalytic efficacy (Shimada et al., 1996b; Ohmori et al., 1998), observed differences in metabolic capacity during development are not surprising (e.g., Lacroix et al., 1997). Finally, given the abundance and importance of CYP3A4/3A5 in the adult intestine and the apparent absence of CYP3A7 in fetal extrahepatic tissues (Yang et al., 1994), it would be of considerable interest to examine the developmental expression of CYP3A4/3A5 in the gastrointestinal tract.

**Ontogeny of the Flavin-Containing Monoxygenases**

The flavin-containing monoxygenases (FMO) (EC 1.14.13.8), encoded by a six-member gene family (FMO1–6), are important for the NADPH-dependent oxidative metabolism of a wide variety of xenobiotics containing nucleophilic nitrogen-, sulfur-, selenium-, and phosphorous-heteroatoms. The ontogeny of human hepatic FMO exhibits a switch reminiscent of that observed for the CYP3A subfamily. In two fetal samples of unknown age, Dolphin et al. (1996) demonstrated hepatic FMO1 but not FMO3 mRNA expression. In contrast, FMO3 but not FMO1 transcripts were demonstrated in four adult liver samples. Yeung et al. (2000) observed FMO1 expression at 14.4 ± 3.5 pmol/mg microsomal protein in five fetal liver samples from 14 to 17 weeks gestation whereas FMO3 immunoreactive protein was nondetectable. In adult tissues, hepatic FMO1 was nondetectable whereas expression was readily detectable in the small intestine (2.9 ± 1.9 pmol/mg microsomal protein) and kidney (47.0 ± 9.0 pmol/mg microsomal protein). A more complete picture of human hepatic FMO developmental expression has recently been completed by our own group (Koukouritaki et al., 2002). In 240 liver samples ranging in age from 8 weeks gestation to 18 postnatal years, the highest level of FMO1 expression was observed at 8 to 15 weeks gestation (7.8 ± 5.8 pmol/mg microsomal protein). FMO1 expression subsequently declined during fetal development and was completely suppressed within 3 postnatal days by a mechanism coupled to birth, but not gestational age. FMO3 expression was observed at low levels between 8 and 15 weeks gestation, but not in subsequent time periods of fetal development. The onset of FMO3 postnatal expression was highly variable. Most individuals failed to express FMO3 in the neonatal period, but expression was detectable by 1 to 2 years of age. Intermediate FMO3 expression was observed until 11 years of age (12.7 ± 8.0 pmol/mg microsomal protein), at which time, a gender-independent increase was observed from 11 to 18 years of age (26.9 ± 8.6 pmol/mg microsomal protein). Although similarities between the CYP3A and FMO temporal switches are apparent, a fundamental difference is observed during the neonatal period. Because the decline in
CYP3A7 expression is accompanied by a simultaneous increase in CYP3A4/3A5, net CYP3A expression remains relatively constant. In contrast, the rapid suppression of FMO1 within 72 postnatal hours and the delayed onset of FMO3 expression results in a null hepatic FMO phenotype in the neonate. The developmental expression of FMO in extraparenchymal tissues remains unknown.

Ontogeny of the Alcohol Dehydrogenases

In contrast to the studies on the cytochromes P450 and FMO, few studies have appeared on the developmental expression of other phase I DME. An exception is alcohol dehydrogenase (ADH) (EC 1.1.1.1). The seminal and pioneering work of Smith et al. (1971) provided definitive evidence for the progressive expression of the three class 1 enzymes, ADH1A (ADHα), ADH1B (ADHβ), and ADH1C (ADHγ), during development. These investigators examined the expression of ADH in liver, lung, kidney, and intestine from 222 individuals ranging in age from 9 weeks gestation to greater than 20 years postnatal age. In fetal liver samples with a mean gestational age of 11 weeks, only the ADH1A enzyme was detectable. By 17 weeks, both ADH1A and ADH1B were measurable, although ADH1A predominated. By 19 weeks, products from all three loci were observed, with ADH1A greater than ADH1B and ADH1B greater than ADH1C. At 30 weeks, ADH1A and ADH1B levels were equivalent but still greater than ADH1C, however, by 36 weeks, ADH1B expression dominated. In the adult, hepatic ADH1A expression was non-detectable, whereas expression from the ADH1B and ADH1C loci were equivalent. Interestingly, this progressive change in expression was tissue-specific. In lung, there were no observed differences between the fetal and adult samples and only ADH1C was detectable. ADH expression in the intestine and kidney was low and did not change appreciably with age. These results are largely in agreement with a more recent study in which steady-state concentrations of the different ADH class transcripts were measured by Northern blot analysis (Estonius et al., 1996). In two different fetal liver samples of unknown age, ADH1A, ADH1B, and ADH1C transcripts were observed in one, whereas ADH1B and ADH1C transcripts dominated in the second. Differing from the earlier report by Smith et al. (1971), ADH1A transcripts dominated in the lung, whereas in the fetal kidney, only transcripts for either or both ADH1B and ADH1C were present. Class I ADH transcripts were present in most adult tissues with the exception of brain, kidney, and placenta. Of the other ADH enzymes, ADH2 (class II, ADHπ) and ADH5 (class V, ADHε) transcripts were observed only in fetal liver at a concentration similar to that seen in the adult. Faint signals also were observed in the adult small intestine and pancreas. Similar to the class I transcripts, ADH3 (class III, ADHγ) transcripts were widely distributed, being detectable at approximately the same concentration in all fetal and adult tissues examined with the possible exception of the brain. In this tissue, the ADH3 transcripts appeared higher in the fetal than in the adult brain, although the signal was readily detectable in both tissues. Given that the ADH1 enzymes are the most efficient ethanol-metabolizing ADH, their absence in placenta and fetal brain would contradict a substantial role for this enzyme family in local ethanol development of central nervous system toxicity.

Summary

Most reports on the developmental expression of the phase I DME have limited their studies to short time frames of development and many have depended on a small number of tissue samples. Furthermore, previous research generally focused on hepatic expression during fetal development and in the postnatal time period. Yet, expression in other tissues is important. There also is a paucity of information regarding changes during early childhood or at puberty, which may account for some of the observed differences between the fetus and adult. Continued efforts using in vitro studies as described herein, complemented by data from pediatric clinical trials utilizing enzyme-selective therapeutic agents will hopefully begin to address this knowledge gap. However, even with the amount of information available, it is apparent that the temporal, tissue-specific, and interindividual variation in phase I DME expression will have a profound impact on xenobiotic biotransformation and disease susceptibility.

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