Developmental Expression of Human Hepatic CYP2C9 and CYP2C19

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ABSTRACT

The CYP2C subfamily is responsible for metabolizing many important drugs and accounts for about 20% of the cytochrome P450 in adult liver. To determine developmental expression patterns, liver microsomal CYP2C9 and -2C19 were measured (n = 237; ages, 8 weeks gestation-18 years) by Western blotting and with diclofenac or mephenytoin, respectively, as probe substrates. CYP2C9-specific content and catalytic activity were consistent with expression at 1 to 2% of mature values (i.e., specific content, 18.3 pmol/mg protein and 79; specific activity, 549.5 pmol/mg/min and 72) during the first trimester, with progressive increases during the second and third trimesters to levels approximately 30% of mature values. From birth to 5 months, CYP2C9 protein values varied 35-fold and were significantly higher than those observed during the late fetal period, with 51% of samples exhibiting values commensurate with mature levels. Less variable CYP2C9 protein and activity values were observed between 5 months and 18 years. CYP2C19 protein and catalytic activities that were 12 to 15% of mature values (i.e., specific content, 14.6 pmol/mg and 20; specific activity, 18.5 pmol/mg/min and n = 19) were observed as early as 8 weeks of gestation and were similar throughout the prenatal period. CYP2C19 expression did not change at birth, increased linearly over the first 5 postnatal months, and varied 21-fold from 5 months to 10 years. Adult CYP2C19 protein and activity values were observed in samples older than 10 years. The ontogeny of CYP2C9 and -2C19 were dissimilar among both fetal and 0- to 5-months postnatal samples, implying different developmental regulatory mechanisms.

The cytochromes P450 (EC 1.14.14.1) comprise a superfamily of heme-thiolate enzymes of which over 2000 individual members are known representing species from all biological kingdoms (see Nelson database at http://drnelson.utmem.edu/CytochromeP450.html). In humans, 17 gene families exist, three of which (CYP1, CYP2, and CYP3) encode enzymes involved in xenobiotic oxidative metabolism. Individual cytochrome P450 enzymes belonging to these families are characterized by diverse substrate specificity and significant differences in regulation, the latter resulting in temporal-, tissue-, and gender-dependent expression patterns (Gonzalez, 1990). Genetic variability among individual family members further contributes to significant intersubject differences in metabolic capacity and pharmacological response (Rogers et al., 2002).

Studies on temporal-specific cytochrome P450 expression during ontogeny have been hampered by the low levels of enzyme generally present in the developing organism, overlapping substrate specificity that makes metabolic phenotyping difficult to interpret, and the paucity of suitable tissue samples for study. For individual cytochrome P450 enzymes, a limited number of studies suggest that expression during ontogeny is highly variable and, among different cytochrome P450 enzymes within the same subfamily, often asynchronous (reviewed in Hines and McCarver, 2002). This temporal asynchrony raises important questions about the specific maturation of biotransformation pathways during ontogeny and the ability of an individual to respond to xenobiotics during this time period.

The human CYP2C subfamily contains four highly homologous genes, CYP2C8, -2C9, -2C18, and -2C19, located in an approximate 500-kbp cluster on chromosome 10q24. The
CYP2C subfamily accounts for about 18% of the total adult liver cytochrome P450 content (Shimada et al., 1994), the major form being CYP2C9 followed by CYP2C19 and CYP2C8 (Goldstein et al., 1994; Edwards et al., 1998). Low levels of CYP2C mRNA and protein have also been detected in the small intestine and other extrahepatic tissues (Klose et al., 1999). Transcriptional regulation of both CYP2C9 and -2C19 is known to involve the constitutive androstane receptor (CAR) and pregnane X receptor (PXR) as well as the glucocorticoid receptor (GR) (Ferguson et al., 2002; Gerbal-Chaloin et al., 2002; Chen et al., 2003). Clinically relevant genetic variability within the CYP2C locus has also been well documented (Goldstein, 2001).

Members of the CYP2C subfamily account for the metabololism of about 20% of clinically important drugs (Goldstein, 2001), including the anticoagulant warfarin (Kaminsky and Zhang, 1997), the H₂ receptor antagonist omeprazole (Andersson et al., 1993), the antiepileptic agent phenytoin (Giancarlo et al., 2001), the antihyperglycemia sulfonylureas tolbutamide (Wester et al., 2000) and glipizide (Kidd et al., 1999), and many nonsteroidal anti-inflammatory agents (Leemann et al., 1993). The CYP2C enzymes also metabolize the endogenous compound arachidonic acid and, as such, may play an important physiological role via the generation of bioactive eicosanoids (Capdevila et al., 1992).

Previous studies of human hepatic CYP2C developmental expression showed no detectable protein or catalytic activity between 16 and 40 weeks of gestation, although low levels of mRNA were observed in fetal tissue. Enzyme levels and parallel activity were greater in the first month after birth, reaching adult levels by 1 year of age, suggesting that CYP2C maturation depends only on postnatal age (Treluyer et al., 1997). However, these studies did not describe the developmental expression pattern of individual CYP2C isoforms. Given the differences in substrate specificity between the different CYP2C enzymes, such knowledge would improve the ability to predict age-related differences in the efficacy of therapeutic entities and the morbidity of numerous toxicants. The objectives of the current study were to characterize the developmental expression pattern for human hepatic CYP2C9 and CYP2C19 and determine the overall interindividual variation in CYP2C9 and CYP2C19 expression as a function of age using the largest set of fetal and pediatric liver samples collected to date (Koukouritaki et al., 2002).

Materials and Methods

Materials. Polyclonal antibodies against recombinant purified CYP2C9 and CYP2C19 were elicited in New Zealand White rabbits as previously described (Sadeque et al., 1993). Horseradish peroxidase-conjugated goat anti-rabbit IgG, nitrocellulose membrane, and enhanced chemiluminescence Western blotting kits were purchased from Amersham Life Science (Arlington Heights, IL). Benchmark precasted protein molecular weight standards were obtained from Invitrogen (Carlsbad, CA). The micro bicinchoninic acid protein assay reagent kit was purchased from Pierce Biotechnology, Inc. (Rockford, IL). Diclofenac (sodium salt) was purchased from Sigma-Aldrich (St. Louis MO). (S)-Mephénytoin, 4′-hydroxymephénytoin, and 4′-hydroxydiclofenac were purchased from BD Gentest (Woburn, MA). All other reagents were obtained from commercial sources at the purest grades available.

Tissue Samples. Frozen specimens of human liver were obtained from the Brain and Tissue Bank for Developmental Disorders, University of Maryland at Baltimore and University of Miami (National Institute for Child Health and Human Development, NOI-HD-8-3283 and NOI-HD-8-3284, respectively), and the Central Laboratory for Human Embryology at the University of Washington (National Institute of Child Health and Human Development, HD-00836). Donor characteristics and exclusion criteria were as described previously (Koukouritaki et al., 2002). Tissue was stored at −80°C until used for the preparation of microsomal suspensions. This study was approved by the Children’s Hospital of Wisconsin and the Medical College of Wisconsin Institutional Review Boards. As a control for the metabolic assays, pooled adult human liver microsomes were obtained from BD Gentest.

Preparation of Microsomes, Electrophoresis, and Immunoblotting. Liver microsomes were prepared by differential centrifugation and analyzed for CYP2C9- and CYP2C19-specific content essentially as described previously (Koukouritaki et al., 2002). Briefly, 40 to 60 μg of microsomal protein from each liver sample were fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, using 10% resolving gels, along with 10, 25, 50, 100, 250, and 500 fmol of recombinant human CYP2C9 and CYP2C19 (Hainje et al., 1996). Both the 100 and 250 fmol CYP2C19 primary antibodies were used at a final concentration of 5 μg/ml diluted in Tris-buffered saline (25 mM Tris, pH 7.5, and 150 mM sodium chloride) containing 0.5% nonfat dry milk. Horseradish-conjugated goat anti-rabbit IgG secondary antibody was diluted 1:5000 in Tris-buffered saline containing 0.5% nonfat dry milk. All incubations for both primary and secondary antibodies were performed for 90 min at room temperature. In all blots, bands corresponding to the protein of interest (CYP2C9 or CYP2C19) were identified by reference to the recombinant CYP2C9 and CYP2C19 and molecular weight standards. After determining the integrated optical densities of the digitized, immunoreactive bands (Kodak Digital Science ID Software; Eastman Kodak, Rochester, NY), the CYP2C9- and CYP2C19-specific content of the patient samples was determined by linear regression based on a standard curve determined from the recombinant CYP2C9 and CYP2C19 included on each blot (GraphPad Instat version 3.00; GraphPad Software, Inc., San Diego, CA). The limit of sensitivity of this assay was 10 fmol of CYP2C9 or CYP2C19.

Metabolic Assays. For both CYP2C9 and CYP2C19 activity determinations, incubations were performed in duplicate and under conditions previously determined to yield linear production of the respective marker metabolite (Stevens et al., 1997). For all incubations, 96-well format cluster tubes (Corning Glassworks, Corning, NY) were precooled and kept on ice until initiating incubations. A mixture of cold substrate and buffer (155 μl total) was added at appropriate concentrations to each tube. A 25-μl aliquot of each microsomal sample (12.5 μg protein/ml final concentration) was transferred to individual tubes, and after preincubating at 37°C for 3 min, reactions were initiated by the addition of 20 μl of 10 mM NADPH. No NADPH was added to human liver microsome negative controls or standards. After 30 min, reactions were stopped by the addition of 50 μl of cold acetonitrile. Samples were immediately placed on ice, capped, and stored at −20°C. In preparation for analysis, plates were warmed to room temperature and centrifuged at 2500 rpm. Supernatant fractions were aspirated, transferred to a clean 96-well plate, and capped for analysis. The conversion of diclofenac (20-μM final assay concentration) to 4-hydroxydiclofenac was used to measure CYP2C9 metabolic activity in individual microsomal preparations. The product, 4-hydroxydiclofenac, was separated and quantified by high-performance liquid chromatography using a previously described method (Fan et al., 2003). Sample areas were read from a linear regression of known standard amounts (range, 40–5000 pmol) using no weighting. Calculations to activity (picomole(s) per minute per milligram) and averaging of replicates were performed in Microsoft Excel. Diclofenac 4-hydroxylase activity in pooled adult human liver microsomes (BD Gentest) was 1340 ± 260 pmol/min/mg protein (n = 42 determinations).
The conversion of (S)-mephenytoin to 4-hydroxymephenytoin was used to measure CYP2C19 metabolic activity in individual microsomal samples. The reaction product, 4-hydroxymephenytoin, was detected and quantified by high-performance liquid chromatography/mass spectrometry as previously described (Fan et al., 2003). Sample areas were read from a linear regression of known standards (range, 4.68–150.00 pmol) using 1/\text{H}1003 weightings. Activities were calculated [picomole(s) per minute per milligram], and replicates were averaged in Microsoft Excel. (S)-Mephenytoin 4-hydroxylation activity in pooled adult human liver microsomes (BD Gentest) was 44.0 ± 10.0 pmol/min/mg protein (n = 41 determinations).

**Data Analysis.** Differences in CYP2C9- and CYP2C19-specific content among different age groups, as well as possible differences between the current study and specific activities previously reported, were assessed using the Mann-Whitney U test. Differences between fetal and neonatal samples (0–30 days postnatal age) were tested using the Mann-Whitney U test. Differences between CYP2C9- and CYP2C19-specific content in individuals within age groups were examined using a paired Student’s t test (GraphPad Software, Inc.). Linear regression was used to evaluate factors contributing to variation in CYP2C expression (SpsPC software; SPSS Inc., Chicago, IL). In all cases, p < 0.05 was accepted as indicating a significant difference.
Results

Developmental Expression Patterns for CYP2C9 and CYP2C19. Western blotting with antibodies to CYP2C9 or CYP2C19 revealed immunoreactive bands with apparent molecular masses near 50 kDa, corresponding to full-length CYP2C9 or CYP2C19, respectively. Figure 1, A and B depict representative Western blots with human hepatic microsomes of different ages. Both CYP2C9 (Fig. 1A) and CYP2C19 (Fig. 1B) proteins were present at low levels in some fetal liver samples but appeared to be much higher in postnatal liver samples. Coefficients of determination ($r^2$) for the standard curves ranged between 0.96 and 0.99 (median, 0.98 for both CYP2C9 and CYP2C19; $n = 21$), and coefficients of variation for independent experiments were 8% for CYP2C9 ($n = 5$) and 6% for CYP2C19 ($n = 5$). The less intense band with a mobility between CYP2C9 and CYP2C19 (some samples are shown in Fig. 1B) is due to cross-reactivity of the CYP2C19 polyclonal antibody with CYP2C8. However, this signal did not interfere with the quantitation of either CYP2C9 or CYP2C19.

The fetal through early infancy developmental expression patterns for CYP2C9 and CYP2C19 are shown in Figs. 2A and 3A, respectively, whereas the expression patterns for all postnatal samples (1 day–18 years) are shown in Figs. 2B and 3B, respectively. Relative to mature values, low CYP2C9 protein levels were detected in many fetal samples between 8 and 24 weeks of gestation. Progressively greater values were observed during the remaining gestational period, such that some samples had specific contents of 10 to 12 pmol/mg, commensurate with values in the mature range (Fig. 2A). CYP2C19 also was detectable at 8 weeks of gestation, but unlike CYP2C9, CYP2C19-specific content was similar among most gestational age samples (Fig. 3A). The CYP2C9 and CYP2C19 developmental expression patterns also were quite different after birth. CYP2C9-specific content was significantly greater in neonatal samples (first 30 days after birth) than in fetal samples older than 24 weeks of gestation (Mann-Whitney $U$, $p < 0.05$); however, variability was extensive over a constant range during the first 5 postnatal months (Fig. 2A). In contrast, CYP2C19 protein levels were not significantly greater in the neonatal versus fetal samples (Mann-Whitney $U$, $p > 0.05$) but, rather, exhibited a somewhat linear increase over the first 5 months postnatal age ($r^2 = 0.477$) (Fig. 3A). It appeared that several individual tissue samples within the perinatal grouping defined a subset with elevated CYP2C19-specific content (Fig. 3A, open circles). Of interest, each of these samples was from individuals who were born prematurely (<33 weeks). An examination of CYP2C9-specific content in all postnatal samples (1 day–18 years, $n = 166$) (Fig. 2B) revealed that most samples from individuals 1 to 2 years postnatal age exhibited mature protein levels. Furthermore, it was apparent that many individuals expressed CYP2C9 at levels equivalent to the maximum observed in the data set immediately after birth. In contrast, with the exception of one or two outliers, CYP2C19 maximum expression levels were not observed in individuals until after 5 months postnatal age. The delay in CYP2C19 expression is also apparent in 18 samples (33% of total) between 5 months and 10 years that have values similar to those of fetal samples (Fig. 3B, hatched circles).

The developmental expression patterns for both CYP2C9 and CYP2C19 also were examined by measuring specific marker activities in individual microsomal preparations. Using CYP2C9-catalyzed diclofenac 4-hydroxylation (Tang et al., 1999), a pattern consistent with that observed by Western blotting was observed (Table 1). Similarly, the conversion of mephenytoin to 4-hydroxymephenytoin was used as a metabolic marker for CYP2C19 (Goldstein et al., 1994), and, again, a pattern consistent with protein measurements was observed (Table 2). However, individual CYP2C9- and CYP2C19-specific contents and activities did not correlate (data not shown). Known coding region polymorphisms that result in reduced activity, but not reduced protein levels (for examples, see review by Goldstein, 2001), may be a contributing factor to this lack of correlation. However, the lack of correlation was not a reflection of postmortem degradation and inactivation of the enzymes. The postmortem interval (time between death and freezing of liver samples) was from 1 to 41 h ($n = 214$; median, 17 h). Neither specific content and postmortem interval nor specific activity and postmortem interval were correlated ($r^2 = 0.004$ and 0.109, respectively). Furthermore, excessive degradation was not seen on any of the Western blots (see Fig. 1 as an example).

The diclofenac 4-hydroxylase and (S)-mephenytoin 4'-hy-
droxylase activity in the oldest age brackets were compared with previously reported data. Diclofenac 4-hydroxylase-specific activity in the oldest age bracket (>5 months-18 years) was significantly lower than that reported by Yasar et al. (2001) or from the data available from BD Gentest (http://www.bdbiosciences.com) (Table 3). In contrast, the (S)-mephenytoin 4-hydroxylase-specific activity in the oldest age

Fig. 3. Developmental expression pattern of CYP2C19. A scatter plot analysis of CYP2C19-specific content as a function of gestational age (weeks) is depicted, along with the transition at birth and the neonatal and early infancy periods (A). The coefficient of determination ($r^2$) was calculated using GraphPad Instat (GraphPad Software, Inc.) after excluding nine outliers (open and hatched circles) based on a residual analysis. A scatter plot analysis of CYP2C19-specific content as a function of postnatal age (years) is depicted (B). Samples from individuals greater than 5 months postnatal age with specific content commensurate with fetal samples are depicted by the hatched circles. Specific content was determined based on the density of the respective immunoreactive bands and a linear regression analysis based on the standard curve determined from the recombinant protein included on each blot (see Fig. 1 as an example).

TABLE 1
Ontogeny of CYP2C9 diclofenac 4-hydroxylase metabolic activity

<table>
<thead>
<tr>
<th>Age Bracket</th>
<th>Sample Size</th>
<th>%ND</th>
<th>Median-Specific Activity $^a$</th>
<th>Specific Activity Range $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8–40 wks</td>
<td>48</td>
<td>60</td>
<td>143</td>
<td>19–254</td>
</tr>
<tr>
<td>25–40 wks</td>
<td>16</td>
<td>38</td>
<td>195</td>
<td>37–735</td>
</tr>
<tr>
<td>Postnatal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 5 mo</td>
<td>88</td>
<td>16</td>
<td>899</td>
<td>53–4600</td>
</tr>
<tr>
<td>&gt;5 mo to 18 yr</td>
<td>71</td>
<td>14</td>
<td>628</td>
<td>60–1906</td>
</tr>
</tbody>
</table>

$^a$ Values for those samples that exhibited detectable activity.

TABLE 2
Ontogeny of CYP2C19 (S)-mephenytoin 4-hydroxylase metabolic activity

<table>
<thead>
<tr>
<th>Age Bracket</th>
<th>Sample Size</th>
<th>%ND</th>
<th>Median-Specific Activity $^a$</th>
<th>Specific Activity Range $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8–40 weeks</td>
<td>64</td>
<td>55</td>
<td>1.3</td>
<td>0.1–345.5</td>
</tr>
<tr>
<td>Postnatal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 5 mo</td>
<td>88</td>
<td>30</td>
<td>6.1</td>
<td>0.1–1516.5</td>
</tr>
<tr>
<td>&gt;5 mo to 10 yr</td>
<td>52</td>
<td>12</td>
<td>22.4</td>
<td>0.1–656.5</td>
</tr>
<tr>
<td>&gt;10 yr to 18 yr</td>
<td>19</td>
<td>0</td>
<td>18.5</td>
<td>0.4–101.7</td>
</tr>
</tbody>
</table>

$^a$ Values for those samples that exhibited detectable activity.
two postnatal phases (Fig. 5). In the mature samples (5
developmental expression was divided into two prenatal and
Results of this analysis are shown in Figs. 5 and 6. CYP2C9
within a group but maximize differences between groups.

different developmental stages, the data presented in Figs. 2
quantify the interindividual variability in expression during
Expression.

be divided into three phases. From 5 months to 10 years postnatal age, CYP2C19-specific content was greater than earlier age brackets (Kruskal-Wallis, \( p < 0.05 \)) but remained highly variable (21-fold range between 5th and 95th percentiles) and significantly lower than mature values (Kruskal-Wallis, \( p < 0.05 \)).

The 5th to 95th percentile values of CYP2C19-specific content in mature samples ranged from 8.1 to 20.8 pmol/mg microsomal protein (4-fold). CYP2C9-specific content was observed in 84% of the samples from 8 to 24 weeks of gestation but was only 1 to 2% of mature values. CYP2C9-specific content was variable (34-fold range between 5th and 95th percentiles). During the neonatal and early infancy period (up to 5 months), 51% of the samples had CYP2C9 protein values that were commensurate with mature values, but again, expression was highly variable (35-fold range between 5th and 95th percentiles).

Factors Impacting CYP2C9 and CYP2C19 Developmental Expression. In adult liver samples, CYP2C9 has been shown to be present at higher levels than CYP2C19 (Goldstein et al., 1994; Edwards et al., 1998). This relationship holds for postnatal samples across development (Fig. 4, B and C), but the reverse is true in fetal samples (Fig. 4A). Comparing all fetal samples (\( n = 71 \)), CYP2C19-specific content was significantly greater than CYP2C9-specific content (paired \( t \) test, \( p < 0.0001 \)). In contrast, when comparing all samples in the early infancy period (0–5 months, \( n = 92 \)) or those samples 5 months or older (\( n = 74 \)), CYP2C9-specific content was significantly greater than CYP2C9-specific content (paired \( t \) test, \( p < 0.0001 \) for both). No relationship existed between values of CYP2C9 and -2C19 during either the fetal or 0- to 5-months postnatal age groups (linear regression, \( r^2 < 0.05 \) for both), consistent with different factors regulating the ontogeny of both proteins. Finally, CYP2C9 and -2C19 content and activity in postnatal samples were each related to age (stepwise linear regression, \( p < 0.05 \) for each) but were not related to gender, ethnicity, or postmortem interval (stepwise linear regression, not significant).

Interindividual Variation in CYP2C9 and CYP2C19 Expression. To better summarize the different phases of CYP2C9 and CYP2C19 developmental expression as well as quantify the interindividual variability in expression during different developmental stages, the data presented in Figs. 2 and 3 were divided into age groups to minimize differences within a group but maximize differences between groups. Results of this analysis are shown in Figs. 5 and 6. CYP2C9 developmental expression was divided into two prenatal and two postnatal phases (Fig. 5). In the mature samples (5 months–18 years), the 5th to 95th percentile values of CYP2C9-specific content ranged from 7.9 to 28.7 pmol/mg microsomal protein (4-fold). CYP2C9-specific content was observed in 84% of the samples from 8 to 24 weeks of gestation but was only 1 to 2% of mature values. CYP2C9-specific content was greater from 25 to 40 weeks of gestation (Kruskal-Wallis, \( p < 0.05 \)), with a median value 27% of that seen in the mature samples, but was variable (34-fold range between 5th and 95th percentiles). During the neonatal and early infancy period (up to 5 months), 51% of the samples had CYP2C9 protein values that were commensurate with mature values, but again, expression was highly variable (35-fold range between 5th and 95th percentiles).

Discussion

This report is the first to document CYP2C9 and -2C19 protein and catalytic activity in the prenatal human liver as well as describe the ontogeny of these two individual gene products. Measurements of both CYP2C9-specific content and catalytic activity are consistent with hepatic expression of this enzyme at 1 to 2% of mature values during the first trimester of fetal development, with progressive increases in expression during the second and third trimesters to levels

\[ \text{TABLE 3} \]

Comparisons of CYP2C9 (S)-mephenytoin 4-hydroxylase metabolic activity

<table>
<thead>
<tr>
<th>Data Source</th>
<th>Sample Size</th>
<th>Median Age</th>
<th>Median-Specific Activity</th>
<th>Specific Activity Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current study &gt;5 mo to 18 yr</td>
<td>61</td>
<td>4.9</td>
<td>628</td>
<td>60–1996</td>
</tr>
<tr>
<td>Yasar et al. (2001)</td>
<td>11</td>
<td>NA</td>
<td>1555</td>
<td>217–4781</td>
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<tr>
<td>BD Gentest(^{bc})</td>
<td>12</td>
<td>47</td>
<td>2515</td>
<td>1020–3970</td>
</tr>
</tbody>
</table>

\(^{a}\) Values for those samples that exhibited detectable activity.
\(^{b}\) http://www.bdbiosciences.com/discovery_labware/gentest/products/.
\(^{c}\) Current study vs. BD Gentest, \( p < 0.05 \).

\[ \text{TABLE 4} \]

Comparisons of CYP2C19 (S)-mephenytoin 4-hydroxylase metabolic activity

<table>
<thead>
<tr>
<th>Data Source</th>
<th>Sample Size</th>
<th>Median Age</th>
<th>Median-Specific Activity</th>
<th>Specific Activity Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current study &gt;10 to 18 yr</td>
<td>19</td>
<td>15.2</td>
<td>18.5</td>
<td>0.4–101.7</td>
</tr>
<tr>
<td>Chiba et al. (1993)</td>
<td>14</td>
<td>NA</td>
<td>55.5</td>
<td>15.0–173.0</td>
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<tr>
<td>Goldstein et al. (1994)(^{bc})</td>
<td>15</td>
<td>NA</td>
<td>145.0</td>
<td>12.0–388.0</td>
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<tr>
<td>Pearce et al. (1996)</td>
<td>9</td>
<td>51</td>
<td>38.4</td>
<td>6.9–86.5</td>
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<tr>
<td>Wrighton et al. (1993)</td>
<td>13</td>
<td>29.5</td>
<td>66.0</td>
<td>2.0–310.0</td>
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<tr>
<td>Heyn et al. (1996)</td>
<td>14</td>
<td>NA</td>
<td>39.5</td>
<td>9.0–319.0</td>
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<tr>
<td>BD Gentest(^{ac})</td>
<td>10</td>
<td>46.0</td>
<td>26.5</td>
<td>4.0–320.0</td>
</tr>
</tbody>
</table>

\(^{a}\) Values for those samples that exhibited detectable activity.
\(^{b}\) Excluded single outlier values with specific activity > 600 pmol/min/mg.

Current study vs. Goldstein et al. (1994), \( p < 0.001 \) (Kruskal-Wallis); differences among all other groups were not significant.
CYP2C9 and CYP2C19 developmental expression

The CYP2C9 protein and catalytic activities that were 12 to 15% of mature values were observed in hepatic tissue samples as early as 8 weeks of gestation. However, in contrast to the gestational increases in CYP2C9, CYP2C19 expression was similar among all gestational ages. The postnatal CYP2C19 expression pattern also was dissimilar to that of CYP2C9, with no significant change in expression between the fetal and immediate neonatal periods. A small subset (n = 5) of neonatal samples exhibited greater than expected CYP2C19-specific content for this age group. Each of these samples was from a premature patient. We speculate that this elevated expression may have been due to the usual clinical practice of administering maternal glucocorticoids during preterm labor to enhance fetal lung maturation. Such a speculation is consistent with the reported existence of a glucocorticoid-responsive element controlling CYP2C19 expression (Chen et al., 2003). CYP2C19 protein values increased somewhat linearly over the first 5 months of postnatal age, suggesting that birth is not sufficient for full postnatal expression. CYP2C19 expression was highly variable from 5 months to 10 years, also consistent with differences in developmental factors that may or may not have a genetic component. A significant but modest increase in CYP2C19-specific content was observed from 10 years through 18 years postnatal age that approached previously reported adult values.

Treluyer et al. (1997) previously reported that CYP2C was not detectable in human fetal liver between 16 and 40 weeks. Moreover, CYP2C expression was reported to be extremely low in newborn liver, greater in the first month after birth, and, between 3 and 12 months, only 30% of adult levels, suggesting that maturation of CYP2C expression depended largely on postnatal factors. Possible differences between this report and the current study that might contribute to the inconsistent results include sample size within specific age brackets, sample quality, and assay sensitivity. A major limiting factor in comparing the two studies is that the report by Treluyer et al. (1997) was limited to an aggregate CYP2C expression pattern. The current data are consistent with a more recent study by Nagata et al. (2003) in which expression profiling was used to examine differential gene expression between the human fetal and adult liver. CYP2C9 mRNA levels were detected in 17- to 37-week fetal liver samples (n = 7) and were elevated 13-fold in postnatal samples (ages 5–65 years, n = 14).

The median CYP2C19-specific content in the oldest age bracket of the current study, 14.9 pmol/mg microsomal protein (n = 20; median age, 15.2 years), was not significantly different from values available from BD Gentest (median, 25 pmol/mg; range, 6.0–49.0; n = 5) (http://www.bdbiosciences.com/) or those predicted by extrapolating from previously reported CYP2C19.1. (S)-Mephenytoin 4-hydroxylase turnover (6.17 ± 0.24 nmol/min/nmol; Goldstein et al., 1994) and the adult liver microsomal (S)-mephenytoin 4-hydroxylase-specific activities reported by Wrighton et al. (1993) (Kruskal-Wallis ANOVA, p > 0.05). Consistent with this observation, the (S)-mephenytoin 4-hydroxylase-specific activities of the pediatric samples in the oldest age bracket of the current study (>10–18 years) was not significantly different from most of the previously reported values (Table 4) and is consistent with the activity determined in a sample of

![Fig. 4. Individual CYP2C9- and CYP2C19-specific content. A scatter plot correlating CYP2C9- and CYP2C19-specific contents in individual liver samples is shown. A, fetal samples (8–40 weeks of gestation); B, neonatal and early infancy samples (birth–5 months postnatal age); and C, samples greater than 5 months–18 years. The line of identity is shown for each age group. The significance of the observed difference was determined using a paired Student's t test.]
pooled adult liver microsomes obtained from BD Gentest (44 ± 10 pmol/min/mg).

In contrast to what was observed with CYP2C19, the observed CYP2C9-specific content in the oldest age bracket (>5 months–18 years; n = 79; median, 5.0 years) of 18.0 ± 6.0 pmol/mg microsomal protein is significantly less than the total CYP2C-specific content of 60 ± 27 pmol/mg protein (n = 60; median age, 54.5 years) reported by Shimada et al. (1994) (ANOVA, p < 0.001). However, the maximal CYP2C9-specific content determined in the current study may not reflect true adult values; i.e., an increase in mean activity would occur with age. Such a possibility is supported by the study of Treluyer et al. (1997), which reported a CYP2C-specific content in liver samples from 3- to 12-month-old individuals that was one-third the CYP2C-specific content observed in adult samples. A further increase in CYP2C9 expression with age...
is also supported by the significantly lower diclofenac 4-hydroxylase activities in the oldest age bracket of the current study (>5 months–18 years) compared with the data available from BD Gentest and the data reported by Yasar et al. (2001) (Table 3). Significantly lower activity in these same pediatric samples is also noted when compared with the mean activity of 2300 ± 100 pmol/min/mg microsomal protein reported by Tang et al. (1999) for 10 pooled adult liver microsomal samples (age not specified) as well as the value measured for pooled adult microsomes (BD Gentest) in the current study, 1340 ± 260 pmol/min/mg microsomal protein.

The reported dominant expression of CYP2C9 over CYP2C19 in adult liver (Goldstein et al., 1994) is in agreement with the postnatal data presented in the current study, although the degree of difference is not as striking. However, our data are consistent with an expected increase in mean CYP2C9, but not CYP2C19, expression with age (see discussion above). Such a change would result in a further increase in the CYP2C9/CYP2C19 ratio depicted in Fig. 4C. Of interest, the inverse relationship is observed in prenatal samples (i.e., CYP2C19 represents the dominant enzyme in most samples). Multiple factors have been implicated in regulating these two genes. Both CYP2C9 and CYP2C19 are known to be regulated by PXR, CAR, and GR (Ferguson et al., 2002; Gerbal-Chaloin et al., 2002; Chen et al., 2003). However, only the proximal CYP2C19 CAR binding sites was found to be active, in contrast to both the distal and proximal CAR binding sites being functional in CYP2C9. This observation has been offered as a probable cause for lower constitutive CYP2C19 expression in adult liver (Goldstein et al., 1994) and, as such, may have a role in regulating fetal and neonatal hepatic CYP2C9 expression. Members of the hepatocyte nuclear factor (HNF) family of transcription factors that regulate hepatic-specific gene expression during development (Cereghini, 1996) have also been shown to be important for regulating several CYP2C subfamily members (Ibeana and Goldstein, 1995) and, as such, may have a role in regulating both fetal CYP2C9 and CYP2C19 expression. In the mouse, it has recently been reported that HNF4α also can regulate the induction of genes encoding xenobiotic metabolizing enzymes by controlling hepatic PXR transcription, and that the up-regulation of PXR during fetal development is due to an induction in HNF4α expression (Kamiya et al., 2003). If a similar role is demonstrated in humans, HNF4α may directly regulate CYP2C9 and CYP2C19 developmental expression as well as indirectly regulate these two genes through its action on PXR. However, such regulation would not explain the differences in relative CYP2C9 and CYP2C19 expression between the fetal and postnatal periods. HNF4α has also shown to be an important modulator of PXR- and CAR-dependent CYP3A4 induction in the liver (Tirona et al., 2003) and, as such, may be playing a similar role for CYP2C expression.

The maturation of organ systems during fetal, neonatal, and childhood periods exerts a profound effect on drug disposition. Accordingly, effective and safe drug therapy in neonates, infants, and children requires a thorough understanding of the dynamic ontogeny of drug absorption, distribution, metabolism, and excretion. The current study demonstrates that CYP2C9 and CYP2C19 expression patterns were highly dissimilar among fetal, neonatal, and early infancy samples, consistent with different mechanisms regulating the developmental expression of these two genes. An important contribution of developmental factors is also supported by the greater interindividual variation in CYP2C9 and CYP2C19 expression observed during the neonatal and early infancy periods.

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