Short Communication

Metabolism of 17 α -Hydroxyprogesterone Caproate, an Agent for Preventing Preterm Birth, by Fetal Hepatocytes

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ABSTRACT:

Preterm delivery (i.e., delivery before 37 completed weeks of gestation) is a major determinant of neonatal morbidity and mortality. Until recently, no effective therapies for prevention of preterm birth existed. In a recent multicentered trial, 17 α -hydroxyprogesterone caproate (17-OHPC) was shown to reduce the rate of preterm birth by 33% in a group of high-risk women. Limited pharmacologic data exist for this drug.

Previous studies have shown that CYP3A is involved in the metabolism of 17-OHPC. In this study, we evaluated the metabolism of 17-OHPC in adult and fetal human hepatocytes and in expressed cytochrome P450 enzymes. 17-OHPC was metabolized by ex-

pressed CYP3A7 and by fetal hepatocytes. The metabolite profile was qualitatively different between expressed CYP3A4 and CYP3A7. Expressed CYP3A4 demonstrated a significantly higher (>10 times) capacity to metabolize 17-OHPC than CYP3A7. Based on retention times, two unique metabolites were observed in the fetal and adult hepatocyte systems along with one common metabolite. The intrinsic clearance of 17-OHPC by fetal hepatocytes was observed to be one-half of that in adults. In summary, this study demonstrates that fetal hepatocytes and, in particular, the fetal form of CYP3A (i.e., CYP3A7) can metabolize 17-OHPC.

Fetal drug exposure is an unavoidable risk associated with drug therapies in pregnant subjects. The role of metabolism, via phase I and II pathways, in regulating fetal exposure is relatively lesser known. Fetal metabolism and the presence of cytochromes P450 (P450s) in the fetal liver have been previously documented in the literature (Ackermann and Richter, 1977; Aranda et al., 1979; Rollins et al., 1979; Rane and Tomson, 1980; Wiebkin et al., 1985; Komori et al., 1990; Chiba et al., 1997; Ladona et al., 2000).

17 α -Hydroxyprogesterone caproate (17-OHPC) is a synthetic steroid analog that is administered during pregnancy (from the second trimester onward) to prevent preterm birth. It is administered as a weekly intramuscular injection of 250 mg. Because almost all lipid-soluble xenobiotics enter the fetal system through placental transfer, there is a high probability that 17-OHPC will penetrate through the placental barrier and gain access to the fetal circulation. Studies performed by using the dual perfused placental lobule technique have shown that 17-OHPC is transferred to the fetal circulation (Yan et al., 2008). Furthermore, analysis of fetal cord blood has shown significant 17-OHPC levels (S. Sharma, R. Venkataramanan, and S. C. Strom, unpublished data), thus confirming the transplacental transport from the mother to the fetus.

Although teratology studies have indicated the safety of 17-OHPC in rodents and nonhuman primates, differences in drug

metabolism between animals and humans are well known. For example, the drug-oxidizing capacities have been reported to develop relatively early in human fetuses, whereas this capability is only reported postnatally in experimental animals, especially non-primates (Rane and Tomson, 1980). Thus, it is important to investigate the pathway(s) involved in the metabolism of 17-OHPC in both the adult and fetal liver. Furthermore, the major pathways by which the fetus can eliminate drugs and their metabolites include placental diffusion and active transport back to the maternal circulation. Because the permeability of placenta to relatively polar metabolites is lower, these metabolites may accumulate in the fetus; especially in the liver where the drug is metabolized. This process may be disadvantageous to the fetus if these metabolites induce cellular toxicity or interfere with the various hepatic transport processes in the liver and kidney. Thus, studies are needed to identify the metabolites of drugs administered during pregnancy and their concentrations present in fetal circulation. Identification of the metabolic pathways will help to make informed decisions regarding drug therapy for pregnant women.

The objectives of the study presented here are to 1) investigate the metabolism of 17-OHPC in fetal hepatocyte cultures, 2) identify the major enzyme and metabolic pathway(s) involved, and 3) compare the fetal kinetics and metabolism of 17-OHPC with primary adult human hepatocyte cultures.

Materials and Methods

Chemicals. 17 α -Hydroxyprogesterone caproate (mol. wt. 428.6) was a gift from Diosynth Inc. (Chicago, IL). Medroxyprogesterone acetate and NADPH were purchased from Sigma-Aldrich (St. Louis, MO). Methanol and water [high-performance liquid chromatography (HPLC) grade] were purchased from Thermo Fisher Scientific (Waltham, MA). Eagle's minimum essential medium (EMEM), Hanks' balanced salt solution (HBSS),

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ABBREVIATIONS: P450, cytochrome P450; 17-OHPC, 17 α -hydroxyprogesterone caproate; HPLC, high-performance liquid chromatography; EMEM, Eagle's minimum essential medium; HBSS, Hank's balanced salt solution; HMM, hepatocyte maintenance medium; DMEM, Dulbecco's modified Eagle's medium; LC-MS, liquid chromatography-mass spectrometry; MRM, multiple reaction monitoring.

hepatocyte maintenance medium (HMM), and Dulbecco's modified Eagle's medium (DMEM) were purchased from Lonza Walkersville, Inc. (Walkersville, MD). Microsomes that were derived from baculovirus-infected insect cells that expressed human P450 isoforms (CYP3A4, 3A5 and 3A7) were purchased from BD Gentest (Woburn, MA).

Expressed Enzyme Microsomal Incubations. Expressed CYP3A4/3A7 (15 pmol) were incubated with 100 μ M 17-OHPC and 10 mM MgCl_2 in 0.1 M phosphate buffer, pH 7.4. The final volume of 0.25 ml was allowed to equilibrate in a shaking water bath for 5 min at 37°C. The reaction was initiated with the addition of NADPH (1 mM). After 60 min of incubation, the reaction was stopped by adding 0.25 ml of ice-cold methanol. The mixture was centrifuged at 3000 rpm for 20 min, and supernatant was injected into the HPLC. The final concentration of methanol was less than 2%. To elucidate the kinetics of 17-OHPC metabolism, incubations of expressed CYP3A7 were carried out by using the above-mentioned method with increasing concentrations of 17-OHPC (0–200 μ M). All of the experiments were performed in duplicate.

Tissue Procurement. Fetal livers, which had a gestational age between 18 and 23 weeks, were obtained from planned termination of pregnancy procedures. The tissues were obtained with informed consent under an Institutional Review Board (IRB) protocol approved by the Human Research Review Committee of the University of Pittsburgh. Liver tissue was immediately placed in chilled EMEM medium, which contained 0.05% antibiotics and antimycotics, 10,000 units/ml penicillin, 25 μ g/ml Fungizone, and 10,000 μ g/ml streptomycin (Invitrogen, Carlsbad, CA), until the cell isolation could be performed (within 0.5–2 h). Adult human liver tissue was procured under an IRB-approved protocol and with support from the liver tissue cell distribution system.

Hepatocyte Isolation Procedure. *Fetal hepatocyte isolation.* The fetal hepatic tissue was scraped away from the biliary tree with a sterile scraper in a 100-mm-diameter cell culture dish. HBSS (30 ml) containing 0.1 M EGTA was added to the scraped tissue and pipetted up and down several times. The cell suspension was subsequently transferred to a 50-ml falcon tube, centrifuged at 100g for 5 min, and the supernatant was poured off. Cell pellet was then washed with 30 ml of HBSS and again centrifuged using the same settings. The supernatant was poured off and the volume of pellet was recorded. The pellet was then resuspended in 25 ml of EMEM (at 37°C), which contained 1 mg/ml collagenase XI, 0.2 mg/ml DNase I, and penicillin/streptomycin. Cell suspension was rolled in a revolving rotary shaker (90 rpm) at 37°C for approximately 25 to 30 min. Tissue was redispersed by pipetting up and down with a 10-ml pipette. The suspension was centrifuged again at 100g for 5 min. The cell pellet was washed thrice with EMEM, and the pellet was resuspended in 40 ml of cell culture media (DMEM+) containing 0.1 μ M insulin, 0.1 μ M dexamethasone, and 25 μ g/ml Fungizone. The suspension was filtered by using a 100- μ m strainer, and cell viability was estimated by using the trypan blue method. The cells were diluted to the desired concentration with DMEM+, and 10% bovine calf serum was added. The cells were plated on collagen-coated plates, and media were changed to serum-free DMEM+ after 2 h. Fetal cells were plated at a density of 1 million cells/well on a 6-well plate and at a density of 0.26 million cells/well on a 12-well plate. The plates were incubated for 4 days to allow cells to grow to confluence before starting the experiments.

Adult hepatocyte isolation. Adult hepatocytes were isolated and plated by using the method previously described by Strom et al. (1996).

Hepatocyte Incubations. Adult and fetal hepatocytes were incubated with 17-OHPC (5 μ M methanolic solution) in HMM for clearance studies. Samples were taken at different time points (15–120 min) to assess 17-OHPC metabolism. Kinetic analysis in fetal hepatocytes was evaluated by incubating different 17-OHPC concentrations (0–80 μ M methanolic solutions) in HMM with cells for 60 min. Control cultures were exposed to HMM containing methanol alone. The final concentration of methanol in all incubations was 0.1%. The samples were stored at –80°C until analysis by liquid chromatography-mass spectrometry (LC-MS).

Analytical Procedure. *Liquid chromatography-tandem mass spectrometry.* A Waters (Milford, MA) HPLC system was used for the analysis of 17-OHPC and its metabolites. Separation was performed on a Waters C18 Symmetry (3.5 μ m, 2.1 $\times$ 150 mm) analytical column at 40°C with a Waters C18 Symmetry (2.1 $\times$ 10 mm) guard column. The mobile phases used were as follows: A, 5%

methanol in water containing 0.1% formic acid; and B, methanol containing 0.1% formic acid. The total run time was 45 min at a flow rate of 0.2 ml/min. A gradient profile was used starting from a mobile phase that contained 50% solution (A), increased linearly to 95% (B), and then returned to the initial condition of 50% (B) to achieve the baseline.

Analysis was performed on a Micromass Quattro Micro triple quadrupole mass spectrometer (Waters) with positive electrospray ionization mode. Medroxyprogesterone acetate was used as the internal standard. For all analytes and the internal standard, the following mass spectrometry settings were used: capillary voltage 3.5 kV; source temperature 100°C; desolvation temperature 300°C; cone gas flow, 50 l/h; desolvation gas flow, 550 l/h; argon pressure, 20 $\pm$ 10 psig; nitrogen pressure, 100 $\pm$ 20 psig. The LC-MS system was controlled, and data were collected by using Masslynx software (version 4.1; Waters).

Full mass scan was performed from 100 to 700 Da at a scan rate of 1 scan/s. Based on the results of the full scan and previously published reports (Sharma et al., 2008; Yan et al., 2008), selection ion monitoring was conducted at m/z 429 (17-OHPC), m/z 445 and 467 ($[\text{M}+\text{OH}]^+$ and its Na^+ adduct), m/z 461 and 483 ($[\text{M}+2\text{OH}]^+$ and its Na^+ adduct), and m/z 477 and 499 ($[\text{M}+3\text{OH}]^+$ and its Na^+ adduct). The results were used in the characterization of the kinetics of 17-OHPC and its metabolites.

Monitoring of extracted ions was also conducted for the following molecular ions: m/z = 313.25 (progesterone) and 329.25 (monohydroxy progesterone). This procedure enabled partial elucidation of the 17-OHPC metabolite structure in adult and fetal hepatocytes and its comparison with previously reported data in adult liver microsomes (Yan et al., 2008).

Data Analysis. Data are expressed as the mean $\pm$ S.D. Apparent kinetic parameters for 17-OHPC metabolism were estimated by nonlinear least-square regression analysis using GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, CA). Half-life estimates were obtained by noncompartmental analysis using WinNonlin 4.1 (Pharsight, Mountain View, CA). Intrinsic clearance was calculated by using the following equation: $\text{Cl}_{\text{int}} = (\ln 2/t_{1/2}) \times (\text{ml incubation}/10^6 \text{ cells})$.

Results

Metabolism of 17-OHPC by Human Fetal Hepatocytes. Incubation of 17-OHPC with fresh human fetal hepatocytes (Fig. 1A) generated three major metabolites (M1-17.60, M2-17.86, and M3-19.18), which were identified by using LC-MS. Incubation of 17-OHPC with fresh human adult hepatocytes (Fig. 1B) also generated three major metabolites (M2-17.84, M4-16.55, and M5-16.84). Metabolite M2 was common to adult and fetal hepatocytes. Two metabolites (M1 and M3) were generated by fetal hepatocytes that were either absent or below the detection limit in the adults. All of the metabolites observed (full scan and selection ion monitoring) in the adult and fetal hepatocyte-based incubations displayed the same m/z ratio (m/z = 445.0). A significant amount of sodium adduct formation (m/z = 467.0) was observed at the same retention time as the major metabolites (M1–M3). Analysis of the fragmentation mass spectra [multiple reaction monitoring (MRM)] in adult and fetal hepatocyte incubations depicted the presence of only 445.0 $\rightarrow$ 313.25 transition in all the major metabolites (M1–M5). Small amounts of dihydroxy metabolites (m/z = 461) were also detected in fetal and adult hepatocytes.

Metabolism of 17-OHPC by Expressed CYP3A7. Incubation of 17-OHPC with baculovirus-expressed CYP3A7 (60 pmol/ml) also generated three (M1, M2, and M3—similar to human fetal hepatocytes) major metabolites. The major metabolites accounted for 90% of the total substrate (17-OHPC) metabolized by the enzyme. Furthermore, studies were carried out to identify and compare the metabolites generated by expressed CYP3A4. The metabolites—M2, M4, and M5 (similar to adult human hepatocytes)—were generated by expressed CYP3A4. The common metabolite [based on a similar retention time that was observed in both CYP3A isoforms (3A4 and 3A7)] was M2, with a m/z value of 445.0. Metabolites (M1 and M3) generated by CYP3A7 were observed to have a similar m/z value (445.0) as that of CYP3A4 metabolites

FIG. 1. LC-MS chromatograms of 17-OHPC and its metabolites derived from incubation of 17-OHPC with fresh human fetal and adult hepatocytes. A, incubation of 17-OHPC with fresh human cultured fetal hepatocytes. The incubation generated six metabolites, three of which were the major metabolites (M1, M2, and M3). In this analytical condition, the retention times of 17-OHPC and its metabolites M1, M2, and M3 were 26.45, 17.60, 17.86, and 19.18 min, respectively. B, incubation of 17-OHPC with fresh human cultured adult hepatocytes. The incubation generated three major metabolites (M2, M4, and M5). In this analytical condition, the retention times of 17-OHPC and its metabolites M4, M5, and M2 were 26.43, 16.55, 16.84, and 17.84 min, respectively. C, incubation of 17-OHPC in media without hepatocytes. No metabolites were generated. The retention time of 17-OHPC was 26.41.

(M4 and M5); however, the relative retention times were different in both cases and, thus, were concluded to be fetal-specific metabolites. Furthermore, a significant sodium adduct ($m/z = 467.0$) was observed at the same retention time as the major metabolites (M1–M3). Analysis of the fragmentation mass spectra (MRM) depicted the presence of only $445.0 \rightarrow 313.25$ transition in all the major metabolites (M1–M5) generated by expressed CYP3A4 and CYP3A7. Small amounts of dihydroxy metabolites ($m/z = 461$) were also detected with expressed CYP3A7 and CYP3A4.

Relative Metabolic Capacities of CYP3A Isoforms. To evaluate the relative 17-OHPC-metabolizing capabilities of CYP3A isoforms (CYP3A4 and CYP3A7), incubations of 17-OHPC (100 μ M, equimolar quantities) were carried out in individually expressed enzyme systems. A comparison of the isoforms' 17-OHPC-metabolizing capability showed that CYP3A7 metabolized $\approx 7\%$ of the substrate at a rate of 3.2 pmol/min/pmol CYP3A7. CYP3A4 metabolized $\approx 83\%$ of the total 17-OHPC added to the incubation at a rate of 47.1 pmol/min/pmol CYP3A4. The results were calculated based on estimating the metabolism of 17-OHPC (loss of parent drug) during incubations.

Furthermore, a comparison of the amount of major metabolites generated (M1–M5) by various isoforms was done. The major metabolites (M1+M2+M3) generated by the CYP3A7 isoform were ≈ 5 times less than those observed for the CYP3A4 (M2+M4+M5) isoform. The relative amount of metabolites observed in the chromatograms (Fig. 1) indicated that the CYP3A7-generated metabolites are likely to be significant enough (compared with CYP3A4 isoforms) to have a potential biological role.

Estimation of 17-OHPC Clearance in Fetal Human Hepatocytes in Comparison with Adult Human Hepatocytes. The time course of 17-OHPC metabolism in freshly isolated human fetal and adult hepatocytes has been illustrated in Fig. 2A. 17-OHPC was incubated for a total period of 120 min, and samples were taken at various time points. At each sampling time point, the amount of unmetabolized 17-OHPC decreased and corresponded to an increase in the total number of major metabolites generated (Fig. 2B). The drug-depletion profiles at 5 μ M depicted a linear log concentration decline, thus showing that the metabolism follows first-order kinetics under these conditions. The result has been reported in terms of 17-OHPC equivalents for the metabolites

FIG. 2. Metabolism of 17-OHPC and generation of major metabolites by fetal (dashed line, $n = 4$) and adult hepatocytes (solid line, $n = 4$). Fresh fetal and adult hepatocytes were incubated with 17-OHPC ($5 \mu\text{M}$), and all three metabolites were quantified by sampling at various time points. The graph depicts depletion of 17-OHPC with time corresponding to a similar increase in the metabolite levels. The major metabolites generated have been expressed in terms of 17-OHPC equivalents. A, the graphic depicts the amount of 17-OHPC (expressed as percentage remaining) metabolized as a function of incubation time. B, the graphic depicts the amount of major metabolites generated by fetal hepatocytes [M1 (*), M2 (●), and M3 (◆)] and adult hepatocytes [M2 (□), M4 (△), and M5 (○)] as a function of incubation time.

because the identity of the metabolites has not been confirmed. Similar profiles were observed in both fetal and adult hepatocytes, although the amount of metabolites varied depending on the metabolic efficiency of individual cases. The half-life and intrinsic clearance for the drug depletion in the case of fetal hepatocytes (66 ± 40 min and 0.045 ± 0.03 ml/min/million cells) was not significantly different from that observed for adult hepatocytes (57 ± 31 min and 0.051 ± 0.03 ml/min/million cells).

Kinetics of 17-OHPC Metabolism by Fetal Human Hepatocytes. The kinetics of metabolism of 17-OHPC was characterized in human fetal hepatocytes ($n = 4$). The rate of 17-OHPC metabolism was linear up to 60 min at a cell density of 1×10^6 hepatocytes/ml. Kinetics of 17-OHPC was evaluated by using the rate of generation of metabolite expressed in terms of 17-OHPC equivalent. An incubation time of 60 min was used because all three metabolites were readily detectable beyond this time point in hepatocytes. M2 was the major metabolite followed by M3 and M1. The generation of all three major metabolites of 17-OHPC by fetal hepatocytes displayed Michaelis-Menten kinetics (Table 1). Michaelis-Menten kinetics was also observed for the major metabolites generated by expressed CYP3A7, and the calculated kinetic parameters are reported in Table 1.

Discussion

The present study shows that 17-OHPC is metabolized by hepatocytes obtained from human fetuses in the second trimester of gestation. This report adds to and confirms the existing data on the ability of the human fetus to metabolize some drugs administered during

TABLE 1

Kinetics of 17-OHPC metabolism

The data represent mean $\pm$ S.D.

Metabolite Numbers	$V_{\max}$ nmol/min/million cells	$V_{\max}$ nmol/min/pmol P450	K_m μM
Fetal_M1	0.46 ± 0.16		12.3 ± 5.1
Fetal_M2	0.50 ± 0.11		11.7 ± 5.3
Fetal_M3	0.65 ± 0.10		25.2 ± 11.0
Adult_M2	1.3 ± 0.52		32.60 ± 49.4
Adult_M4	0.35 ± 0.23		33.10 ± 25.1
Adult_M5	0.79 ± 0.50		38.80 ± 25.7
CYP3A7_M1		0.0103 ± 0.002	1.8 ± 0.90
CYP3A7_M2		0.0104 ± 0.001	1.7 ± 0.50
CYP3A7_M3		0.0130 ± 0.002	5.2 ± 2.1
CYP3A4_M2		0.20 ± 0.02	57.5 ± 14.7
CYP3A4_M4		0.04 ± 0.006	40.1 ± 16.1
CYP3A4_M5		0.032 ± 0.003	10.5 ± 4.8

Fetal, fetal hepatocytes ($n = 4$); adult, adult hepatocytes ($n = 4$); CYP3A7, expressed CYP3A7 ($n = 2$); CYP3A4, expressed CYP3A4 ($n = 2$).

pregnancy (Ackermann and Richter, 1977; Aranda et al., 1979; Rolins et al., 1979; Chiba et al., 1997; Ladona et al., 2000).

Incubation of 17-OHPC with fresh human fetal hepatocytes in culture generated three major metabolites (M1, M2, and M3), which had a similar m/z values of 445.0 but different retention times. These m/z values suggest the formation of monohydroxylated metabolites ($429 + 16 = 445$), which were isomers hydroxylated at different carbon positions. Metabolism of 17-OHPC by expressed CYP3A7 to three major metabolites (M1, M2, and M3) provided the first indication that CYP3A7 is involved in the metabolism of 17-OHPC. In addition, the metabolism of 17-OHPC by CYP3A4 to form M2, M4, and M5 ($m/z = 445.0$) confirmed the results reported by Yan et al. (2008), wherein they identified the metabolites using adult human liver microsomes.

Metabolites (M1 and M3) generated by CYP3A7 were observed to be isoform-specific based on the relative retention times and preliminary fragmentation data. These CYP3A7-specific metabolites are likely to be structural isomers of CYP3A4 metabolites.

Analysis of the fragmentation mass spectra (MRM) in adult/fetal hepatocytes and expressed CYP3A4/3A7 incubations depicted the presence of only $445.0 \rightarrow 313.25$ transition in the major metabolites (M1–M5). Thus, we can conclude that all of the major metabolites generated by the expressed enzyme and hepatocyte incubations had substitutions in the caproate side chain and not in the progesterone ring. These conclusions were in agreement with those observed by Yan et al. (2008) in human liver microsomes. Metabolite structure elucidation that involved “Ion Trap”-based fragmentations and NMR studies are currently being carried out to confirm the structure.

CYP3A7 is the major CYP3A isoform expressed in the fetal liver (Komori et al., 1990; de Wildt et al., 1999). The metabolite profile in fetal hepatocytes was observed to match the profile generated by incubating 17-OHPC with expressed CYP3A7. Furthermore, metabolism of 17-OHPC by fresh adult human hepatocytes generated a metabolite profile that was similar to the profile observed in incubations with expressed CYP3A4. It can be concluded that CYP3A7 expressed in fetal hepatocytes and CYP3A4 expressed in adult hepatocytes form the major pathway in the metabolism of 17-OHPC. Comparison of the two expressed CYP3A isoforms' 17-OHPC-metabolizing capacity showed CYP3A7 to have a much lower metabolic capacity (based on percentage of 17-OHPC metabolized) than CYP3A4. A reverse transcription-polymerase chain reaction-based analysis of mRNA expression in the fetal livers used in our study showed that the CYP3A7 expression level is ~ 100 times higher than that of CYP3A4 (data not shown). Thus, in spite of the lower 17-OHPC-metabolizing capability, the results suggest that CYP3A7 may

play a major role in the fetal disposition of 17-OHPC. Although CYP3A5 has been observed to metabolize 17-OHPC (Sharma et al., 2008) much more efficiently than CYP3A4, it is less likely to play a significant role in fetal metabolism because the expression level is almost negligible (Hakkola et al., 2001).

The relative metabolic capacities of adult and fetal hepatocytes were evaluated by estimating the half-life and intrinsic clearance of 17-OHPC (5 μ M) based on loss of parent drug experiments. The results indicated that adults and fetuses have a similar capacity to metabolize 17-OHPC. The intrinsic clearance ($V_{\max}/K_m$; μ l/min/million cells) for fetal hepatocytes for the major metabolites (M1 = 0.06 ± 0.05 , M2 = 0.07 ± 0.06 , M3 = 0.02 ± 0.01) was also comparable to that calculated for adult hepatocytes (M2 = 0.06 ± 0.03 , M4 = 0.02 ± 0.01 , M5 = 0.04 ± 0.02). Similar results were observed in experiments with expressed CYP3A isoforms. The intrinsic clearance ($V_{\max}/K_m$; μ l/min/pmol P450) for expressed CYP3A7 (M1 = 0.006 ± 0.002 , M2 = 0.006 ± 0.001 , M3 = 0.0025 ± 0.001), the major isoform in fetal liver, was comparable with expressed CYP3A4 (M2 = 0.004 ± 0.001 , M4 = 0.002 ± 0.0003 , M5 = 0.004 ± 0.001), the major isoform in adult liver.

Metabolism of 17-OHPC by human fetal hepatocytes displayed a regular Michaelis-Menten profile. The estimated $V_{\max}$ values for the major metabolites (M1–M3) in fetal hepatocytes were observed to be ~3- to 4-fold lower than those for adult human hepatocytes (M2 – 2.0, M4 – 0.4, M5 – 1.0; data from our laboratory). Similar results were also observed in studies that involved expressed CYP3A4 and CYP3A7. The total number of metabolites generated by incubating 17-OHPC (100 μ M) with CYP3A7 (major isoform in fetal liver) was observed to be ~5-fold less than that with CYP3A4 (major isoform in adult liver).

In summary, the intrinsic clearance of 17-OHPC was observed to be similar in adult and fetal hepatocyte cultures. Furthermore, the kinetics of 17-OHPC metabolism was also observed to be similar in hepatocytes and expressed enzyme systems.

Several factors can affect the kinetics of a drug in fetal hepatocytes. It is known that both adult and fetal livers express a similar set of transporters, which are responsible for the uptake and efflux of various endogenous and exogenous chemicals but differ in their level of expression (Chen et al., 2005; Cizkova et al., 2005). Thus, kinetic differences (or lack thereof) observed between adult and fetal hepatocytes for a drug could be related to the levels of expression of these transport proteins. For example, clearance of a drug by hepatocytes at nonsaturating concentrations (<1 μ M) may be more dependent on the total uptake or other factors that influence the intracellular concentration than on the intrinsic metabolic capacity of the expressed P450s. Other possibilities could include differences in tissue protein binding between adult and fetal hepatocytes.

In this study, we report that human fetal hepatocytes are capable of significantly metabolizing 17-OHPC. CYP3A7 was observed to be the major enzyme involved in the metabolism of 17-OHPC via the oxidative pathway. Fetal-specific metabolites (M1 and M3) were generated, which were not observed in the adult hepatocyte system. All of the major metabolites (M1–M3) generated by fetal hepatocytes were monohydroxylated 17-OHPC compounds with substitutions in the caproate side chain.

17-OHPC has been reported to have a clinical signal for embryo-fetal toxicity in the human trials conducted to date (Christian et al., 2007), and a similar signal has been observed in rhesus monkeys (Hendrickx et al., 1987). However, in the absence of elaborate reproductive toxicology and pharmacokinetic studies in animals and humans, the data are inconclusive to address the embryo-fetal toxicity issues vis-à-vis 17-OHPC and/or its metabolites.

Thus, given the ability of fetal liver to significantly metabolize

17-OHPC, future studies are needed to investigate the effect of accumulation of 17-OHPC or its metabolites, generated by adult and fetal hepatic pathways, on the fetal hepatic elimination processes and any associated embryo-fetal toxicity.

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