Identification of Enzymes Involved in the Metabolism of 17α-Hydroxyprogesterone Caproate: An Effective Agent for Prevention of Preterm Birth

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Abstract:
Preterm delivery, that is delivery before 37 completed weeks of gestation, is the major determinant of neonatal morbidity and mortality. Until recently, no effective therapies for prevention of preterm birth existed. In a recent multicentered trial, 17α-hydroxyprogesterone caproate (17-OHPC) reduced the rate of preterm birth by 33% in a group of high-risk women. Limited pharmacologic data exist for this drug. The recommended dose is empiric; the metabolic pathways are not well defined especially in pregnant women; and the fetal exposure has not been quantified. To define the metabolic pathways of 17-OHPC we used human liver microsomes (HLMs), fresh human hepatocytes (FHHs), and expressed enzymes. HLMs in the presence of NADPH generated three metabolites, whereas two major metabolites were observed with FHHs. Metabolism of 17-OHPC was significantly inhibited by the CYP3A4 inhibitors ketoconazole and troleandomycin in HLM and FHH. Metabolism of 17-OHPC was significantly greater in FHH treated with the CYP3A inducers, rifampin and phenobarbital. Furthermore, studies with expressed enzymes showed that 17-OHPC is metabolized exclusively by CYP3A4 and CYP3A5. The caproic acid ester was intact in the major metabolites generated, indicating that 17-OHPC is not converted to the primary progesterone metabolite, 17α-hydroxyprogesterone. In summary, this study shows that 17-OHPC is metabolized by CYP3A. Because CYP3A is involved in the oxidative metabolism of numerous commonly used drugs, 17-OHPC may be involved in clinically relevant metabolic drug interactions with coadministered CYP3A inhibitors or inducers.

Preterm birth is the major determinant of neonatal morbidity and mortality (Paneth, 1995; Mattison et al., 2001). None of the numerous interventions to reduce the rate of preterm birth including labor-inhibiting drugs or strategies such as home uterine monitoring have proven effective. Recent evidence from the Maternal–Fetal Medicine Units Network showed that weekly injections of 250 mg of 17α-hydroxyprogesterone caproate (17-OHPC) reduced the rate of preterm birth in high-risk women by 33% (Meis et al., 2003). The dose used in this study was not based on the pharmacologic properties of 17-OHPC but rather on empiric doses used in clinical trials in the 1960s and 1970s. 17-OHPC (Fig. 1A) is a synthetic hormone produced by the esterification with caproic acid at the 17 carbon position of the metabolite of the natural female sex hormone progesterone (Fig. 1C). In animal studies, 17-OHPC produces a longer lasting and more robust prostaglandin effect on the endometrium than progesterone (Wu and Allen, 1959). The mechanism of action of 17-OHPC is unknown, but the prostaglandin effect is likely related to its concentration. We have recently reported plasma concentrations in pregnant women with twins treated with 17-OHPC (Caritis and Venkataramanan, 2007), but limited data exist on how this drug is metabolized and whether the drug or its metabolites reach the fetal circulation. The observation that only one third of cases benefit from treatment with 17-OHPC suggests that variability in 17-OHPC metabolism or drug interactions may also contribute to the observed variability in clinical response after a fixed dosing regimen. The purpose of the present study was to identify the various enzymes [cytochromes P450 (P450s) and flavin-containing monoxygenase (FMO) enzymes] involved in 17-OHPC metabolism and to characterize the drug’s metabolites.

Materials and Methods

Chemicals. 17α-OHPC (molecular mass 428.6) was a gift from Diosynth Inc., (Chicago, IL). The radioactive isotope of 17α-hydroxy-[1,2,6,7-3H]progesterone [1-14C]caproate was custom-synthesized by RTI International (Re-

ABBREVIATIONS: 17-OHPC, 17α-hydroxyprogesterone caproate; P450, cytochrome P450; FMO, flavin-containing monoxygenase; HLM, human liver microsome; HPLC, high-performance liquid chromatography; HMM, hepatocyte maintenance medium; FHH, fresh human hepatocyte; LC/MS, liquid chromatography/mass spectrometry; MS/MS, tandem mass spectrometry.
search Triangle Park, NC). Quinidine, sulfaphenazole, coumarin, ketocozale, methimazole, α-naphthoflavone, testosterone, 6β-hydroxytestosterone, and NADPH were purchased from Sigma-Aldrich (St. Louis, MO). Methanol was purchased from Fisher Scientific (Fair Lawn, NJ). Microsomes derived from baculovirus-infected insect cells were purchased from BD-Gentest (Woburn, MA).

**Human Liver Microsomes.** Human liver samples were obtained from the Hepatocyte Transplantation Laboratory at the University of Pittsburgh. Donors of human liver tissue had no history of liver disease, but the liver was not used for transplantation.

**Preparation of Human Liver Microsomes.** Liver pieces were dissected and kept in cold saline on ice. Liver microsomes were prepared by a standard differential centrifugation procedure with minor modifications (Court and Greenblatt, 1997; Nelson et al., 2001). Briefly, liver pieces were homogenized with 3 volumes of a homogenization buffer (50 mM Tris-HCl buffer, 1.0% KCl, and 1 mM EDTA, pH 7.4) using an electrical homogenizer (Polytron, Brinkmann Instruments, Westbury, NY). The crude homogenate was centrifuged (700 relative centrifugal force for 20 min, and supernatant was injected into the high-performance liquid chromatography (HPLC).

Incubations of [3H], [14C]17-OHPC were also performed with HLMs. Radiolabeled 17-OHPC was incubated with three individual human liver microsomal preparations for 10 min using the above-mentioned method, and the samples were analyzed directly by HPLC connected to a radioactivity detector.

**Chemical Inhibition Studies.** Various P450 inhibitors were used in the study to identify P450s that might be involved in the metabolism of 17-OHPC. Human liver microsomal preparations (n = 3) were used for the studies with the incubations being performed in triplicate. Experiments were done at different 17-OHPC concentrations (0–200 µg/ml expressed as 0–467 µM) spanning the concentrations seen clinically to identify the type of inhibition keeping the inhibitor concentration constant. P450 isomeric selective chemical inhibitors were used at the following concentration: CYP1A2 (α-naphthoflavone, 10 µM), CYP2A6 (coumarin, 20 µM), CYP2C9 (sulfaphenazole, 5 µM), CYP2D6 (quinidine, 5 µM), CYP3A (ketocazol, 1.0 µM), and FM303 (methimazole, 200 µM). Additionally, inhibition studies were also carried out by incubating 17-OHPC (25 µM) with different concentrations of ketocazol (0.01–10 µM) to determine the IC50 for the reaction. The formula used to determine the IC50 for microsomal incubations involving 17-OHPC and ketocozale involves estimating percentage inhibition, which was calculated as follows: % Inhibition = [(17-OHPC without inhibitor − 17-OHPC with inhibitor) / (17-OHPC without inhibitor)] × 100, where 17-OHPC without inhibitor is the amount of 17-OHPC metabolized in the absence of ketocozale relative to the total amount of 17-OHPC. 17-OHPC with inhibitor is the amount of 17-OHPC metabolized in the presence of ketocozale relative to the total amount of 17-OHPC. Subsequently, IC50 values, or the inhibition concentration resulting in 50% inhibition of 17-OHPC metabolism, were determined from a plot of the percentage inhibition versus the logarithm of ketocozale concentration.

In the case of mechanism-based inhibitor-like troleandomycin (100 µM), preincubation was done for 30 min at 37°C before adding the substrate (17-OHPC, 25 µM). In inhibition experiments with ketocozale (10 µM), the substrate was coinubated with inhibitor.

**Expressed Enzyme Microsomal Incubations.** The incubations (n = 3) were carried similarly to the method described for HLMs. To evaluate the involvement of P450 isoforms in 17-OHPC metabolism, 20 pmol of each expressed enzyme was incubated for 60 min with 17-OHPC, and the samples were analyzed using HPLC-UV.

**Preparation of Human Hepatocytes.** Hepatocytes were prepared by a three-step collagenase perfusion technique (Strom et al., 1996). Hepatocytes were plated on Falcon six-well culture plates (1.5 × 106 cells/well; Falcon; BD Biosciences Discovery Labware, Bedford, MA) previously coated with rat tail collagen in hepatocyte maintenance medium (HMM) supplemented with 0.1 µM insulin, 0.1 µM dexamethasone, 0.05% streptomycin, 0.05% penicillin, 0.05% amphotericin B, and 10% bovine calf serum. After allowing the cells to attach for 4 to 6 h, medium was replaced with serum-free medium containing all the supplements described above. Cells were maintained in culture at 37°C in an atmosphere containing 5% CO2 and 95% air. After 24 h in culture, unattached cells were removed by gentle agitation, and the medium was changed. The medium was changed every 24 h, and the hepatocytes were maintained in culture for the experiment.

**Incubations with Fresh Human Hepatocytes.** Briefly, hepatocytes were maintained in culture in the presence of the chemical under study or vehicle control (dimethyl sulfoxide 0.1% or MeOH 0.1%). On the day of the experiment, cells were washed with HMM devoid of insulin, dexamethasone, antibiotics, and antifungal drugs. Drug stocks were prepared in methanol at 1000-fold incubation concentration (100 mM). Ten microliters of this 100 mM stock was added to a vial containing 10 ml of HMM. Reactions were started by incubating six-well cell culture plates containing human hepatocytes (1.5 million cells/well) with the drug in HMM solutions for 60 min. At the end of that time, 1 ml of medium was sampled and stored at −80°C analysis. The remaining media were aspirated, and the cells were harvested in phosphate buffer (0.1 M, pH 7.4) and stored at −80°C for protein determination.

For acute inhibition experiments (n = 4), human hepatocytes were coinubated with 17-OHPC (25 µM) in the presence and absence of inhibitors of NADPH. After 60 min of incubation (optimum incubation time), the reaction was stopped by immediately adding equal volume of cold methanol. The mixture was centrifuged at 700 relative centrifugal force for 20 min, and supernatant was injected into the high-performance liquid chromatography (HPLC).
(troleandomycin and ketoconazole). The samples were incubated for 30 min and collected as described above.

The induction experiments were initiated 24 h after plating the cells. The hepatocytes (n = 3) were incubated with the inducers (rifampin, phenobarbital, and clotrimazole) for 4 days before adding HMM/17-OHPC (50 μM) to estimate the effect of CYP3A induction on 17-OHPC metabolism.

**Correlation Studies.** Testosterone 6β-hydroxylation was used as the marker for CYP3A activity (Chiba et al., 1996). Formation rates of 17-OHPC metabolite (M2) were measured using microsomes (n = 7) and fresh human hepatocytes (FHHs) (n = 7) at a substrate concentration of 100 μM. These rates were correlated with 6β-hydroxytestosterone formation activity to assess the involvement of CYP3A isoforms.

**Analytical Procedure.** HPLC-UV. Analysis of the unmetabolized drug and the potential metabolites obtained from HLM-based incubations was performed using an HPLC system equipped with UV detection. The HPLC system comprised an autosampler (712 WISP, Waters, Milford, MA) and solvent delivery system (Waters 501) attached to a UV detector (Waters 486). Chromatography was performed with a 4.6 × 250-mm, 100Å, 5-μm Symmetry C18 Waters column. Isocratic elution was performed with a mobile phase of 90% (v/v) methanol in water at a flow rate of 0.8 ml/min, column temperature of 25°C, and eluent monitored at 242 nm. The intraday and interday variation expressed as coefficient of variation did not exceed 10% in any of the assays.

The concentration of 17-OHPC in samples was quantitated by comparing the peak areas in samples with a standard curve of the pure drug. The metabolites were quantitated by expressing them in terms of 17-OHPC equivalents.

**Liquid chromatography/tandem mass spectrometry.** Samples obtained from FHH-based incubations were analyzed by a liquid chromatography/mass spectrometry (LC/MS) (Thermo Electron, San Jose, CA) system consisting of a Surveyor quaternary LC pump, a Surveyor autosampler, coupled to a triple quadrupole mass spectrometer (TSQ Quantum) and equipped with an atmospheric pressure ionization electrospray interface. Instrument control and data acquisition were performed with the Xcalibur software (Thermo Electron, 2.0). Tandem MS (MS/MS) conditions for the analytes were optimized by pump infusion of 17-OHPC stock solutions using the Quantum Tune Master software (Thermo Electron). The HPLC column used was a Symmetry C18 (150 × 2.1 mm, 3.5 μm, Waters) with an appropriate guard column (10 × 2.1 mm, Symmetry). The mobile phases used were A, water (0.1% formic acid) and B, acetonitrile (0.1% formic acid). The total run time was 35 min at a flow rate of 0.2 ml/min. A gradient profile was used starting from a mobile phase containing 10% solution B, increased linearly to 90% B over first 15 min, and the eluents were monitored at 242 nm. The concentration of the metabolite was quantitated by comparing the peak areas in samples with a standard curve containing known amount of the metabolite.

**Radio-HPLC.** Analytical separations were achieved using conditions similar to the aforementioned HPLC method. The metabolites were analyzed using a radiometric model 525TR/FLO-ONE flow-through radioactivity detector (PerkinElmer Life Sciences, Boston, MA), and peak areas were integrated with Windows-based (Microsoft, Redmond, WA) FLO-ONE version 3.61.

**Data Analysis.** Data are expressed as the mean ± S.D. Student’s t test was used to assess the significance of results. IC50 was calculated using GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, CA).

**Results**

**Metabolism of 17-OHPC by P450s in Human Liver Microsomal and FHH Preparations.** Incubation of 17-OHPC with HLMs resulted in the generation of three (M1–M3HLM) main metabolites wherein (M2HLM) was the major metabolite. The formation of metabolites was observed to increase up to 60 min using a microsomal protein concentration of 0.5 mg/ml. Hence, the abovementioned conditions were used for all the incubations unless specified otherwise. The formation of metabolites (M1HLM and M3HLM) was low compared with M2HLM; thus, accurate data could not be obtained for these minor metabolites, and the article focuses on the major metabolite (M2HLM).

Incubation of 17-OHPC with FHHs resulted in the generation of five metabolites (M1–M5HLM). The major metabolite generated was M2HHL, which was used to characterize 17-OHPC metabolism in FHHs. Figure 2 depicts the chromatograms obtained using LC-UV and MS for analysis. Figure 2, A and B, shows the metabolism of 17-OHPC in microsomal incubates in the presence/absence of NADPH. All the metabolites were formed in an NADPH-dependent manner only. Figure 2C shows the metabolism of 17-OHPC in FHHs incubated for 1 h.

Incubation of radiolabeled 17-OHPC with HLM generated one major and two minor metabolite peaks (data not shown). The recovery of radioactivity from the incubations was 85 to 97%. The major metabolite constituted approximately 60 to 65% of the metabolized 17-OHPC, and the two minor metabolites were estimated to be approximately 15%. The remaining 20% of the metabolite could not be accounted for because of limitations of the analytical method used. The two ([14C, 3H] labels on the 17-OHPC molecule were observed to remain intact in the metabolites generated, confirming that the caproate side chain or the ring structure was not cleaved during metabolism in human livers.

**Metabolic Profiles in HLMs and Hepatocytes.** Metabolism of 17-OHPC was evaluated over time using HLMs and hepatocytes. The concentrations of unmetabolized 17-OHPC and the major metabolite (expressed in terms of 17-OHPC equivalent) were determined over a time period of 0 to 180 min incubation. Figure 3, A and B, shows that the concentration of 17-OHPC decreased in a time-dependent manner, and the concentration of the metabolite increased proportionately. Approximately 60% of the parent drug (17-OHPC) was metabolized within 60 min, and M2HLM accounted for almost 50% of metabolized 17-OHPC.

**Identification of Human P450 Isoforms.** Incubations with HLMs. 17-OHPC incubation with microsomes for 60 min in the presence of various representative inhibitors of P450 isoforms. A similar reaction was performed in the absence of the inhibitors, and the rate of major metabolite (M2HLM) generated was compared between the two conditions to identify the isoforms responsible for 17-OHPC metabolism. As summarized in Fig. 4, α-naphthoflavone, sulfaphenazole, coumarin, quinidine, and methimazole did not inhibit the metabolism of 17-OHPC by the corresponding P450s. In contrast, the amount of M2HLM was significantly decreased in the presence of ketoconazole, indicating that the metabolism of 17-OHPC was markedly inhibited. The Vmax (0.29 ± 0.02 nmol/min/mg) and Km (77.94 ± 19.4 μM) values for M2HLM were observed to decrease significantly in the presence of ketoconazole (Vmax = 0.03 ± 0.002, Km = 15.38 ± 3.9). An IC50 (0.17 μM) value for the inhibition of 17-OHPC metabolism by ketoconazole in HLMs (n = 3) was also calculated (Fig. 5).

**Incubations with baculovirus-expressed human P450 isoforms.** Studies were also performed in baculovirus-infected insect cells expressing various P450s. The metabolizing activity of each CYP3A isoform for 17-OHPC was compared with control microsomes, which were devoid of any P450 activity. The results indicate the involvement of CYP3A4/5 in the metabolism of 17-OHPC and the formation of the major metabolite (M2HLM) was a result of CYP3A4/5 pathway (Table 1).

**Incubations with human hepatocytes: inhibition studies.** Results obtained from inhibition experiments performed in HLM were confirmed in fresh adult human hepatocytes (Fig. 6) using chemical inhibitors for CYP3A (ketocazole and troleandomycin). Troleandomycin and ketoconazole inhibited 17-OHPC metabolism (M2HHL).
formation) by 75 and 89%, respectively, indicating involvement of CYP3A4/5.

Induction studies were performed in FHHs to further confirm CYP3A to be the primary enzyme responsible for 17-OHPC metabolism. CYP3A inducers like rifampin, phenobarbital, and clotrimazole increased M2HH formation (Fig. 7). Rifampin showed the maximum (2.2-fold) induction in 17-OHPC metabolism. Phenobarbital showed 2.1-fold, whereas clotrimazole showed the least, 1.2-fold induction in human hepatocytes.

Correlation studies. Rates of formation of 17-OHPC metabolite (M2) with liver microsomes and fresh adult human hepatocytes were measured at a substrate concentration of 100 μM. There was considerable interindividual variation in the values of metabolite generated (Fig. 8). Formation of M2HLM and M2HH correlated significantly with the testosterone 6-hydroxylation activity (CYP3A4) in both microsomes (r = 0.89) and hepatocytes (r = 0.91), respectively.

Discussion

To the best of our knowledge, this is the first report identifying the human hepatic enzymes, which play a major role in the metabolic absorption peak for 17-OHPC after incubation with FHHs for 1 min. No metabolite formation was detected. The retention time for 17-OHPC was 21.97.
and methimazole (FMO3, CYP3A4). Approximately 80% inhibition of 17-OHPC was observed at 1 μM in H9262 CYP3A isoforms in metabolizing 17-OHPC and the potential involvement of major metabolite formation (M2HLM) expressed in terms of 17-OHPC equivalents.

Inhibition of 17-OHPC metabolism was evaluated by estimating the formation of M2HLM, which is expressed in terms of 17-OHPC equivalents. Results from Table 1 show that ketoconazole significantly inhibited 17-OHPC metabolism. Inducers of CYP3A, namely, rifampicin, phenobarbital, and clotrimazole, significantly increased 17-OHPC metabolism in comparison with control. This indicated the role of CYP3A isoforms in metabolizing 17-OHPC and the potential involvement of CYP3A4. Inhibition of 17-OHPC metabolism was evaluated by estimating the formation of M2HLM. Significant levels of metabolite were detected on incubation of 17-OHPC with M2HLM being the major metabolite. Ketoconazole and troleandomycin (a known CYP3A inhibitor) at 1.0 μM inhibited 90% of 17-OHPC biotransformation in HLMs. These findings and the lack of effect of other P450s. In fresh human adult hepatocytes, five (M1–5HLM) metabolites were observed on incubation of 17-OHPC with M2HLM being the major metabolite. Ketoconazole and troleandomycin (a known CYP3A inhibitor) significantly inhibited 17-OHPC metabolism. Inducers of CYP3A, namely, rifampicin, phenobarbital, and clomipramine, significantly increased 17-OHPC metabolism in comparison with control. Thus, the results in HLMs were successfully reproduced in FHHs. This showed CYP3A to play the major role in 17-OHPC metabolism in hepatocytes as well.

To further confirm our results, we conducted experiments to check compounds would likely coincide. The results obtained confirmed this expectation. In HLMs, 17-OHPC was metabolized to one major and two minor metabolites. P450 inhibition experiments indicated CYP3A4/5 to form the main metabolic pathway. Ketoconazole (CYP3A inhibitor) at 1.0 μM inhibited 90% of 17-OHPC biotransformation in HLMs. These findings and the lack of effect of α-naphthoflavone, quinidine, coumarin, and sulfaphenazole (inhibitors for CYP2A6, CYP2D6, CYP2C8, and CYP2C9) suggest a major role for CYP3A in the metabolism of 17-OHPC.

Identification of P450s involved in the metabolism of 17-OHPC

Table 1 shows the IC50 values for the ketoconazole-mediated inhibition of 17-OHPC (25 μM) metabolism calculated to be 0.17 μM in HLMs (n = 3). Inhibition of 17-OHPC metabolism was evaluated by estimating the formation of M2HLM. No metabolites were observed in the absence of NADPH, thus confirming the metabolism to be P450-mediated. Incubation of 17-OHPC with heat-inactivated microsomes did not result in any loss of 17-OHPC or generation of any 17-OHPC metabolites. Significant metabolism of 17-OHPC was seen in all three systems that were tested. Generation of metabolites and loss of parent drug confirmed this observation. No metabolites were observed in the absence of NADPH, thus confirming the metabolism to be P450-mediated. Incubation of 17-OHPC with HLMs under the conditions for direct generation of conjugates did not alter 17-OHPC concentrations or yield any 17-OHPC metabolites, ruling out a direct role of UDP glucuronosyltransferase-mediated pathway. However, studies evaluating the role of UDP glucuronosyltransferase as a sequential pathway in 17-OHPC metabolism are being carried out currently.

Multiple approaches were used to identify the P450 enzymes involved in the metabolism of 17-OHPC. Metabolism of medroxyprogesterone acetate, a potent progestogenic compound, has been reported in literature (Kobayashi et al., 2000) to be catalyzed mainly by CYP3A4. Because medroxyprogesterone acetate is structurally similar to 17-OHPC, it was expected that the metabolic pathways for both
the ability of expressed enzyme systems to catalyze the biotransformation of 17-OHPC. CYP3A isoforms were the only enzyme systems that metabolized 17-OHPC significantly. The enzyme activity for 17-OHPC metabolism was observed to be higher for CYP3A5 isoform than CYP3A4. The reason for the differential activity is not known at this time.

The CYP3A subfamily is known to be expressed most abundantly (i.e., from 10–60% of total P450s) in human liver and plays a pivotal role in the oxidative metabolism of many clinically important drugs. Among the CYP3A isoforms tested (i.e., CYP3A4 and CYP3A5), CYP3A4 is the major isoform in adult humans. CYP3A5 is polymorphically expressed in approximately 10 to 20% of the adult livers (Wrighton et al., 1990). Overall, we can predict that CYP3A4 would be the major P450 isoform responsible for the hepatic metabolism of 17-OHPC in the majority of adult patients given 17-OHPC. Given that CYP3A5 has higher activity for 17-OHPC, genetic polymorphism in CYP3A5 may have a significant role in 17-OHPC metabolism and pharmacokinetics, but this remains to be evaluated. It has been proposed that the prolonged and more potent action of 17-OHPC over progesterone involves the cleavage of 17-OHPC molecule to 17α-hydroxyprogesterone (Fig. 1B) and release of free caproic acid. It was also suggested that caproic acid could affect genomic pathways and hence have an effect on progesterone signaling pathways (Attardi et al., 2007). However, results of radio-HPLC–based method confirmed that the structure of 17-OHPC remained intact during metabolism by human enzymes. Our observation does not support the hypothesis that 17-OHPC is a prodrug that gets metabolized to progesterone or hydroxyprogesterone (Fig. 1B) and release of free caproic acid. It was also suggested that caproic acid could affect genomic pathways and hence have an effect on progesterone signaling pathways (Attardi et al., 2007). However, results of radio-HPLC–based method confirmed that the structure of 17-OHPC remained intact during metabolism by human enzymes. Our observation does not support the hypothesis that 17-OHPC is a prodrug that gets metabolized to progesterone or hydroxyprogesterone. Incubation of FHHs with 17α-hydroxy progesterone caproate significantly increased the formation of 17α-hydroxyprogesterone (Fig. 1B) and release of free caproic acid. It was also suggested that caproic acid could affect genomic pathways and hence have an effect on progesterone signaling pathways (Attardi et al., 2007). However, results of radio-HPLC–based method confirmed that the structure of 17-OHPC remained intact during metabolism by human enzymes. Our observation does not support the hypothesis that 17-OHPC is a prodrug that gets metabolized to progesterone or hydroxyprogesterone. Incubation of FHHs with 17α-hydroxyprogesterone caproate significantly increased the formation of 17α-hydroxyprogesterone (Fig. 1B) and release of free caproic acid. It was also suggested that caproic acid could affect genomic pathways and hence have an effect on progesterone signaling pathways (Attardi et al., 2007). However, results of radio-HPLC–based method confirmed that the structure of 17-OHPC remained intact during metabolism by human enzymes. Our observation does not support the hypothesis that 17-OHPC is a prodrug that gets metabolized to progesterone or hydroxyprogesterone.

Our study shows that the metabolism of 17-OHPC is predominantly mediated by CYP3A isoforms, mainly CYP3A4. Given that the activity of CYP3A enzyme is known to vary between subjects, one would expect large variation in the pharmacokinetics of 17-OHPC in pregnant subjects. In women pregnant with twins we noted a large variation in concentrations despite a fixed dosing regimen (Caritis and Venkataramanan, 2007). CYP3A4 plays a major role in the metabolism of various drugs because of its abundance in the liver and its broad substrate specificity. Numerous clinically important drugs are known as substrates of CYP3A4 (Rendic and Di Carlo, 1997). Thus, further in vitro and clinical studies are required to assess 17-OHPC–associated clinically relevant metabolic drug interaction with any coadministered CYP3A4 substrates/inhibitors.

Pregnancy is a dynamic state of the human body that is characterized by significant variations in the physiology and metabolism. Changes in the metabolizing activity of P450s, especially CYP3A4, have been reported in literature (Tracy et al., 2005). Activity of CYP3A4 has been shown to increase significantly in all the trimesters in humans. Furthermore, the expression level of CYP3A isoforms varies from individual to individual. Thus, we expect significant interindividual fluctuations in 17-OHPC plasma concentrations in pregnant patients over time. In clinical studies, 17-OHPC is administered as a fixed dose regimen of 250 mg weekly. The therapy was observed to be effective in 33% of the patients in the study of Meis et
It is possible that the low success rate of 17-OHPC may be attributable to the significant variation in CYP3A-mediated metabolism of 17-OHPC in these patients. Based on the abovementioned facts, it may be necessary to investigate various dosing regimens and individualize the therapy with 17-OHPC. Monitoring 17-OHPC plasma levels and adjustment of dose accordingly may be needed to improve therapeutic outcomes.

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References


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