

Isoform-specific regulation of cytochromes P450 expression by estradiol and progesterone

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Abbreviations: b5, NADH-cytochrome b5 reductase; CITCO, 6-(4-Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-3,4-dichlorobenzyl) oxime; CAR, constitutive androstane receptor; CYP, Cytochrome P450; ER, estrogen receptor; HH, human hepatocyte; PXR, pregnane X receptor; POR, P450 oxidoreductase

Abstract

Results from clinical studies suggest that pregnancy alters hepatic drug metabolism in a cytochrome P450 (CYP) isoform-specific manner, and rising concentrations of female hormones are potentially responsible for the changes. The objective of this study was to comprehensively characterize the effects of estrogen and progesterone on the expression and activity of major drug-metabolizing CYPs. To this end, primary human hepatocytes were treated with estradiol and progesterone, and mRNA expression and activity levels of 10 different CYP isoforms were determined. The results showed that estradiol enhances CYP2A6, CYP2B6, and CYP3A4 expression, whereas progesterone induces CYP2A6, CYP2B6, CYP2C8, CYP3A4, and CYP3A5 expression. The induction was mainly observed when the average hormone concentrations were at the levels reached during pregnancy, suggesting that these effects are likely pregnancy-specific. Estradiol also increased enzyme activities of CYP2C9 and CYP2E1 without affecting the mRNA expression levels by unknown mechanisms. Taken together, our results show differential effects of estrogen and progesterone on CYP expression, suggesting involvement of different regulatory mechanisms in female hormone-mediated CYP regulation. Our findings potentially provide a basis in mechanistic understanding for altered drug metabolism during pregnancy.

Introduction

Medication use during pregnancy is prevalent; over 50% of pregnant women take at least one prescription drug (Glover et al., 2003; Andrade et al., 2004). Pregnancy is known to influence the pharmacokinetics of drugs administered to pregnant women. For example, the rate of hepatic drug metabolism changes during pregnancy in a CYP isoform-specific manner; metabolism of CYP2A6, CYP3A4, CYP2C9, and CYP2D6 substrates is increased by ~50%, 100%, 20%, and 50%, respectively, in the 3rd trimester as compared to nonpregnant controls, whereas that of CYP2C19 and CYP1A2 substrates is decreased by 40-50% (Glover et al., 2003; Anderson, 2005a; Hodge and Tracy, 2007; Anderson and Carr, 2009). The factors responsible for the changes in CYP-mediated drug metabolism during pregnancy remain unknown.

Estradiol and progesterone are major female hormones, and their plasma concentrations gradually increase during pregnancy, reaching up to 100 nM and 1 μ M for estradiol and progesterone, respectively, at term. These are significantly higher than the concentrations of estradiol (0.2-1 nM) and progesterone (5-50 nM) during menstrual cycle (Tulchinsky et al., 1972; Mathur et al., 1980; Stricker et al., 2006). Accumulating evidence suggests that female hormones may influence the rate and extent of drug metabolism. For example, changes in hepatic drug elimination are similar for CYP1A2, CYP2A6, and CYP2C19 substrates in pregnant women and users of oral contraceptives (Hodge and Tracy, 2007). Also, the elimination of CYP2A6, CYP2B6, and CYP3A4 substrates is faster in females than in males, whereas CYP1A2-mediated elimination is slower in females (Anderson, 2005b). These directional changes in CYP activity are similar to those occurring during pregnancy. Despite the evidence, whether and how the female hormones modulate expression and/or activities of major hepatic CYP enzymes remains unclear.

The effects of estrogens and progesterone on hepatic CYP expression have been examined in animals (Dean and Stock, 1975; Waxman et al., 1985; Dannan et al., 1986; Ochs et al., 1986; Waxman and Holloway, 2009). In rats, estradiol is known to enhance expression of

female-predominant CYP2C7 and female-specific CYP3A9 (Bandiera and Dworschak, 1992; Wang and Strobel, 1997). On the other hand, progesterone alters the rate of drug metabolism in a substrate-specific manner, suggesting CYP isoform-specific effects of progesterone on drug metabolism (Dean and Stock, 1975; Ochs et al., 1986). Extrapolation of these results to humans, however, is difficult in part due to significant divergence in hepatic drug-metabolizing enzyme genes and apparent differences in regulation of drug-metabolizing enzymes expression between humans and rodents. For example, sexual dimorphism of CYP expression in humans is not as prominent as in rodents.

In MCF-7 (human breast cancer) and HepG2 (human hepatoma) cells, estradiol is shown to enhance expression of CYP2A6 and CYP2B6 while decreasing CYP2C19 expression by activating estrogen receptor α (ER α) (Higashi et al., 2007; Chen et al., 2009; Lo et al., 2010; Mwinyi et al., 2010; Koh et al., 2012); however, the effects of estradiol on expression of other CYPs remain unknown. On the other hand, progesterone has been shown to enhance activities of pregnane X receptor (PXR)-driven promoters in various immortalized cell lines (Lehmann et al., 1998; Masuyama et al., 2003; Jeong et al., 2008). PXR, along with constitutive androstane receptor (CAR), is a ligand-activated nuclear receptor known to mediate upregulation of drug-metabolizing enzymes including CYP2A6, CYP2B6, and CYP3A4 (Burk et al., 2004; Dickins, 2004; Ferguson et al., 2005). Whether progesterone influences expression of other CYP enzymes or whether regulatory mechanisms other than PXR signaling are involved in progesterone actions on CYP expression remains unknown.

The objective of this study was to comprehensively characterize the effects of estrogen and progesterone on the expression of major CYPs using human hepatocytes. Our results show differential effects of estradiol and progesterone on CYP expression in an isoform-dependent manner, which suggests the potential involvement of multiple regulatory mechanisms in CYP regulation by the female hormones.

Materials and Methods

Chemicals and reagents. Acetaminophen, chlorzoxaxone, coumarin, dexamethasone, diclofenac, 17 β -estradiol, ethinylestradiol, indomethacin, ITS, S-mephenytoin, 7-hydroxycoumarin, 4-hydroxymephenytoin, 4-methylumbelliferone, phenobarbital, s-nirvanol, phenytoin, *p*-nitrophenol, *p*-nitrocatechol, phenacetin, phenytoin, progesterone, rifampin, and William's E medium were obtained from Sigma (St. Louis, MO). Midazolam, 1-hydroxymidazolam, d4-acetaminophen and prazepam were purchased from Cerilliant (Round Rock, TX). 4'-Hydroxydiclofenac was purchased from Axxora (San Diego, CA). CITCO, i.e. 6-(4-Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-3,4-dichlorobenzyl) oxime, was purchased from Biomol (Plymouth Meeting, PA). Bufuralol and 1-hydroxybufuralol were purchased from BD Biosciences (Franklin Lakes, NJ). 6-Hydroxychlorzoxazone was purchased from Cayman Chemical (Ann Arbor, MI). Formic acid (ACS grade), acetonitrile (Optima grade), and methanol (Optima grade) were purchased from Fisher Scientific (Pittsburgh, PA).

Primary human hepatocytes culture. Freshly isolated primary human hepatocytes from different donors (age ranging from 29 to 70, no known history of liver diseases, alcohol abuse or hepatitis B or C infection) were obtained from CellzDirect (Pittsboro, NC) or Liver Tissue Cell Distribution System (Pittsburgh, PA). Hepatocytes (1×10^6 cells per well in 12 well plates) were shipped overnight in cold preservation media. Upon receipt, media were replaced with serum-free Williams' E media (without phenol red) containing 0.1 μ M dexamethasone, 10 μ g/ml gentamicin, 15 mM HEPES (pH 7.4), 2 mM L-glutamine, and ITS+. Cells were maintained at 37 $^{\circ}$ C in a humidified incubator with 5% CO₂. After overnight incubation, cells were treated with vehicle control (0.1% ethanol), female hormones [estradiol (1 μ M) or progesterone (10 μ M)] or known inducers [rifampin (10 μ M), CITCO (100 nM), ethanol (100 mM), or phenobarbital (0.5

mM)] for 72 hr. The media were replaced every 6, 12, and 24 hr for estradiol, progesterone, and CYP inducer treatment, respectively.

Determination of estradiol and progesterone concentration in media. Hepatocytes were treated with estradiol (1 μM) or progesterone (1 or 10 μM), and media were sampled at various time points up to 12 hours. Estradiol and progesterone concentrations in culture media were measured by LC/MS/MS (Agilent 1200 HPLC interfaced with Applied Biosystems Qtrap 3200 using an electrospray ion source). Separation was performed with a Zorbax Eclipse XDB-C8 column (4.6 \times 50 mm, 3.5 μm ; Agilent Technologies) at a flow rate of 0.4 ml/min. For measurement of estradiol concentrations, linear gradient of the mobile phase, consisted of water [0.02% (v/v) ammonium hydroxide] and acetonitrile, was used for separation: 15% acetonitrile at time 0 increased to 90% at 7 min. MRM pairs used for estradiol and ethinylestradiol (internal standard) were 271.2/145.1 and 295.2/145.1 respectively. For measurement of progesterone concentrations, linear gradient of mobile phase of water [0.1% (v/v) HCOOH] and acetonitrile was used for separation: 20% acetonitrile at time 0 increased to 80% at 6 min. MRM pairs used for progesterone and prazepam (internal standard) were 315.2/109.2 and 325.0/271.1, respectively. Average concentrations of estradiol or progesterone in media were calculated as follows: $C_{\text{ave}} = \text{AUC}_{0-\tau} / \tau$, where $\text{AUC}_{0-\tau}$ is the area under the curve (hormone concentration vs. time from 0 to τ hr) and τ the media change interval.

RNA isolation and quantitative real time-PCR (qRT-PCR). Total RNAs were isolated from human hepatocytes using Trizol® (Invitrogen, Carlsbad, CA). RNA purity was confirmed by 260:280 nm absorbance ratio (greater than 1.7) and the integrity of ribosomal RNAs was visually confirmed by gel electrophoresis. cDNA was synthesized using High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). Quantitative real-time PCR amplification of cDNA

corresponding to 40 ng of total RNA was performed in a reaction mixture (10 μ L) containing 1X concentration of TaqMan[®] Universal Master Mix (Applied Biosystems) and the following TaqMan[®] Gene Expression Assay (individual assay ID is listed below): CYP1A2 (Hs01070369_m1), CYP2A6 (Hs00868409_s1), CYP2B6 (Hs03044634_m1), CYP2C8 (Hs00258314_m1), CYP2C9 (Hs00426397_m1), CYP2C19 (Hs00426380_m1), CYP2D6 (Hs02576167_m1), CYP2E1 (Hs00559367_m1), CYP3A4 (Hs00430021_m1), CYP3A5 (Hs00241417_m1), CAR (Hs00901571_m1), PXR (Hs00243666_m1), POR (Hs00287016_m1), b5 (Hs00157217_m1), and GAPDH (Hs99999905_m1). Cycling conditions were initial denaturation at 95 °C for 10 min followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 1 min. The relative mRNA expression CYP isoforms upon hormone or inducer treatment was determined by normalizing the gene expression levels to GAPDH ($2^{-\Delta\Delta Ct}$ method).

Determination of CYP activity. Hepatocytes were treated with vehicle control (ethanol), a female hormone, or one of the known inducers for 72 hr, and CYP activities were determined as previously described (Choi et al., 2009; Choi et al., 2011). Briefly, the media were replaced with fresh media containing one of CYP isoform-selective probe substrates for CYP1A2 (phenacetin 50 μ M), CYP2A6 (coumarin 100 μ M), CYP2B6 (S-mephenytoin 100 μ M), CYP2C9 (diclofenac 100 μ M), CYP2C19 (S-mephenytoin 100 μ M), CYP2D6 (bufuralol 60 μ M), CYP2E1 (*p*-nitrophenol 100 μ M), and CYP3A4 (midazolam 15 μ M) and sampled at various time points (30 min to 4 hr). The concentration of a metabolite of each probe substrate was measured by LC/MS/MS (Agilent 1200 HPLC interfaced with Applied Biosystems Qtrap 3200) using an electrospray ion source. The mobile phase consisted of water (0.1% formic acid) and acetonitrile. Separation was performed with a Zorbax Eclipse XDB-C8 column (4.6 \times 50 mm, 3.5 μ m) (Agilent Technologies, Santa Clara, CA) except for acetaminophen and 7-hydroxycoumarin (YMC C8 4.6 \times 150 mm, 3.5 μ m, Waters, Milford, MA). The quantitative analytical conditions

for the metabolites (e.g., internal standards, MRM pairs, and mobile phase gradients) have been previously described (Choi et al., 2009; Choi et al., 2011).

Statistical Analysis

Each experiment (from an individual donor) was performed in triplicates and data are expressed as means \pm standard deviation (S.D.). Statistical difference between hormone-treated vs. vehicle-treated control groups was determined by Student's t-test. For statistical testing among different treatment groups, one-way ANOVA test was performed for multiple comparisons followed by posthoc Dunnett's test.

Results

Estradiol and progesterone are rapidly metabolized by human hepatocytes. Estradiol and progesterone are known to be rapidly eliminated from the body via hepatic metabolism (Goldzieher and Brody, 1990; Kuhl, 1990). In studying the effects of estradiol and progesterone on the expression of major CYPs using human hepatocytes, we first determined elimination rates of estradiol and progesterone in the hepatocyte cultures. Human hepatocytes were treated with estradiol (1 μ M) or progesterone (1 μ M), and hormone concentrations at various time points were measured. The result (Fig. 1A) showed that estradiol was metabolized at a first-order elimination rate by the human hepatocytes (half-life: 0.57 ± 0.12 hr). The average concentration of estradiol over 6 hr was 157 ± 39 nM, which corresponds to the average concentration during the 3rd trimester of human pregnancy (Tulchinsky et al., 1972). Similar results were obtained in different batches of human hepatocytes (data not shown). Based on the results, throughout the study, culture media containing 1 μ M estradiol were replenished every 6 hr during the daytime and 12 hr for overnight incubation to maintain the average estradiol concentration at the level of

the 3rd trimester of pregnancy. Progesterone was also metabolized rapidly by human hepatocytes at a first-order elimination rate (Fig. 1B, half-life: 0.73 ± 0.16 hr). This led to the average concentration of 92 ± 16 nM over 12 hr, which corresponds to the progesterone concentration at the early phase of pregnancy, lower than the concentration at full-term (~ 1 μ M) (Tulchinsky et al., 1972). Accordingly, a ten-fold higher concentration of progesterone (i.e., 10 μ M) was used throughout the study to treat hepatocytes. After treatment with 10 μ M progesterone (Fig. 1C), the estimated average concentration of progesterone over 12 hr in culture media was 3.28 ± 0.47 μ M.

Estradiol induces CYP2A6, CYP2B6, and CYP3A4 expression. To determine the effects of estradiol on CYP expression, human hepatocytes (from five different donors) were treated with estradiol, and the mRNA expression of major drug-metabolizing CYPs (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5) were determined by qRT-PCR. Estradiol increased the expression levels of CYP2A6 (5.9 to 23.4-fold), CYP2B6 (4.8 to 14.4-fold) and CYP3A4 (1.5 to 6.8-fold), but not those of the remaining CYP enzymes we examined (Fig. 2A). Of note, induction of CYP3A4 with estradiol was overall in a smaller magnitude than that of CYP2A6 and CYP2B6 and was observed only in 2 of 5 different batches of hepatocytes. The effects of estradiol on CYP2A6, CYP2B6, and CYP3A4 showed a concentration-dependency (Fig. 2B).

The extent of induction of CYP2A6 and CYP2B6 mRNA levels by estradiol was comparable to that by a prototypical inducer of CYP expression, CITCO (a CAR activator) (Fig. 3A); induction of CYP2A6 mRNA levels by estradiol was 76-530% of the induction by CITCO while the induction of CYP2B6 was 55-78%. On the other hand, the extent of CYP3A4 induction by estradiol was much smaller as compared to that by rifampin (a PXR activator) (Fig. 3A), indicating minor effects of estradiol on CYP3A4 expression. The effects of estradiol on activities of CYP2A6, CYP2B6 and CYP3A4 in comparison with those of known CYP inducers showed results similar to the estradiol effects on mRNA expression of those genes (Fig. 3B); estradiol

increased the activities of CYP2A6 (1.5-60.8 fold), CYP2B6 (1.5-4.9 fold), and CYP3A4 (0.9-2.7 fold) while CITCO and rifampin increased the activities of CYP2A6, CYP2B6 and CYP3A4 by 6.2-14.2 fold, 2.9-7.9 fold, 4.6-7.4 fold respectively. The activities of CYP1A2, CYP2C19, and CYP2D6 were not affected by estradiol treatment (data not shown). Taken together, estradiol enhanced expression of CYP2A6 and CYP2B6 to the level comparable to that by CITCO. Estradiol also enhanced CYP3A4 expression although the effect was variable and the magnitude of induction was small.

Estradiol enhances CYP2C9 and CYP2E1 activities but not their mRNA expression.

Estradiol did not affect mRNA levels of CYP2C9 or CYP2E1 in human hepatocytes (Fig. 2). Also, determination of protein levels of CYP2C9 and CYP2E1, by using western blotting, showed a lack of estradiol effects on protein levels of CYP2C9 and CYP2E1 (data not shown). Interestingly however, estradiol enhanced activities of CYP2C9 (diclofenac 4'-hydroxylation) and CYP2E1 (*p*-nitrophenol hydroxylation) by 1.3-2.0 and 1.7-2.1 fold, respectively, as compared to vehicle treatment in human hepatocytes (Fig. 4). Although the magnitude was small, the induction was comparable to that by known inducers of CYP2C9 (phenobarbital) and CYP2E1 (ethanol) (Roberts et al., 1995; Gerbal-Chaloin et al., 2002). The enhanced activities of CYP2C9 and CYP2E1 upon estradiol treatment were further verified using different probe drugs for the enzymes, tolbutamide and chlorzoxazone. Estradiol increased tolbutamide hydroxylation (CYP2C9) by 1.8-fold, as compared with vehicle treatment, similar to the results obtained using diclofenac as a probe drug (Fig. 4). On the other hand, although the effect of estradiol on chlorzoxazone 6-hydroxylation (CYP2E1) did not reach statistical significance, estradiol increased the activity by 1.4-fold (Fig. 4). Considering that the activities of CYP2C9 and CYP2E1 are sensitive to expression levels of redox partners such as P450 oxidoreductase (POR) or NADH-cytochrome b5 reductase (b5) (Kaminsky and Guengerich, 1985; Wortham et al., 2007), mRNA levels of POR and b5 in estradiol- or vehicle-treated hepatocytes were determined

by qRT-PCR. The expression of POR and b5 was not altered by estradiol (data not shown).

Taken together, our results indicate that estradiol moderately enhances activities of CYP2C9 and CYP2E1 while not affecting their expression levels.

Progesterone induces expression of CYP2A6, CYP2B6, CYP2C8, CYP3A4 and CYP3A5.

To determine the effects of progesterone on expression of CYP enzymes, we treated human hepatocytes with vehicle or progesterone for 72 hr and examined mRNA expression of major CYPs by using qRT-PCR. The results (Fig. 5A) showed that progesterone increased the expression of CYP2A6 (1.7-3.4 fold), CYP2B6 (1.8-7.6 fold), CYP2C8 (1.4-9.2 fold), CYP3A4 (1.4-12.0 fold), and CYP3A5 (1.4-6.7 fold). The effects of progesterone on CYP2A6, CYP2B6, CYP2C8, CYP3A4 and CYP3A5 showed concentration dependency (Fig. 5B). Progesterone did not show significant effects on the mRNA expression or enzyme activities of the remaining CYPs (data not shown).

When compared to the action of prototypical inducers of CYP expression (i.e., CITCO and rifampin), the magnitude of induction in CYP2A6, CYP2B6, and CYP3A4 mRNA levels by progesterone was smaller and exhibited large interindividual-variability (Fig. 6A); induction of CYP2A6 and CYP2B6 by progesterone was 33-74% and 17-54%, respectively, of the induction by CITCO while induction of CYP3A4 was 14-45% of the induction by rifampin. Induction of CYP2C8 and CYP3A5 were comparable to the induction by rifampin (46-108% and 83-107%). Progesterone-mediated changes in activities of CYP2A6, CYP2B6, and CYP3A4 appeared to show patterns similar to those in mRNA expression of the genes (Fig. 6B). Taken together, our data indicate that progesterone enhances expression of CYP2A6, CYP2B6, CYP2C8, CYP3A4 and CYP3A5 although the effects appear minor when compared to those by typical CYP inducers.

Discussion

Results from clinical studies suggest that increased plasma levels of female hormones are potentially responsible for altered drug metabolism during pregnancy. However, there have been no comprehensive studies conducted using human liver tissues for the effects of female hormones on expression and activity of major CYPs. In this study, we characterized the effects of major female hormones, estradiol and progesterone, on the expression of 10 different CYP isoforms in human hepatocytes.

We treated primary human hepatocytes with estradiol and progesterone with frequent media change, and measured the expression and activity levels of different CYPs. Estradiol and progesterone are non-charged lipophilic compounds that can freely cross cell membranes via passive diffusion (Stumpf, 1969; Revelli et al., 1998). Accordingly, we expect that after 72 hr of drug treatment, the concentration of estradiol and progesterone between media and intracellular compartment would reach equilibrium and that the media concentration likely represents the intracellular concentrations of estradiol and progesterone.

Our results showed that estradiol induces the expression of CYP2A6, CYP2B6, and CYP3A4 in human hepatocytes. The induction was mainly observed when the average estradiol concentration was at the level reached during pregnancy, suggesting that the hormonal effects on CYP expression are likely pregnancy-specific. Induction of CYP2A6 by estradiol is in agreement with a previous study where estradiol increased transcriptional activity of CYP2A6 via activation of ER α (Higashi et al., 2007). The magnitude of induction in CYP2A6 mRNA levels by estradiol shown in our study was to a similar extent to that by CITCO, a known inducer of CYP2A6 (Maglich et al., 2003). This suggests that induction of CYP2A6 by estradiol potentially leads to clinically significant outcomes, and may be in part responsible for the reported increases in metabolism of CYP2A6 substrates (e.g., nicotine) during pregnancy (Dempsey et al., 2002). CYP2B6 induction by estradiol was also comparable to that by CITCO, suggesting potential

increases in elimination of CYP2B6 substrates (e.g., bupropion) during pregnancy. Whether pregnancy indeed influences CYP2B6-mediated drug metabolism in humans remains to be determined. Induction of CYP3A4 expression by estradiol was relatively weak when compared to that by rifampin, rendering its clinical significance questionable.

Of interest, estradiol increased CYP2C9 and CYP2E1 activities while not affecting their expression. In estradiol-treated hepatocytes, similar increases in the CYP enzyme activity were observed for multiple probe substrates of CYP2C9 (diclofenac and tolbutamide) and CYP2E1 (*p*-nitrophenol and chlorzoxazone). Furthermore, co-treatment of hepatocytes with a phase II enzyme inhibitor (i.e., salicylamide 2 mM) to block the known glucuronidation of *p*-nitrophenol (Zerilli et al., 1997; Hanioka et al., 2001) did not abolish the increased *p*-nitrophenol hydroxylation upon estradiol treatment (data not shown). These results suggest that potential non-specificity of the substrates unlikely contributes to the enhanced CYP2C9 and CYP2E1 activity by estradiol. Notably, CYP2C9 and CYP2E1 activities are known to be sensitive to the expression levels of redox partners such as POR or b5 (Kaminsky and Guengerich, 1985; Wortham et al., 2007). Our data, however, showed that estradiol does not alter the mRNA expression levels of POR and b5, suggesting that POR and b5 are unlikely responsible for the enhanced CYP2C9 and CYP2E1 activities by estradiol. Previous studies have reported that post-translational modification of CYP enzymes may influence CYP activities without affecting expression levels [reviewed in (Aguar et al., 2005)]. Whether estradiol influences post-translational modification of CYP2C9 and CYP2E1 leading to altered enzyme activity remains unknown.

Progesterone enhanced mRNA expression of CYP2A6 (minor), CYP2B6, CYP2C8, CYP3A4, and CYP3A5. Although this list of CYP isoforms partly overlaps with that by estradiol, the pattern of CYP induction by progesterone was found to be different; for example, progesterone showed the largest effect on CYP3A4 expression whereas estradiol enhanced CYP2B6 expression greatest. This appears to suggest that differential regulatory mechanisms

may play a role in the hormonal action on CYP expression. Indeed, we and other groups have reported that estradiol is an activator of human CAR (but not of PXR) whereas progesterone is an activator of PXR (but not of CAR) at concentrations reached during pregnancy (Lehmann et al., 1998; Jeong et al., 2008; Koh et al., 2012). Expectedly, estradiol-activated ER α would also play a role in the differential actions of estradiol and progesterone on CYP expression; CYP2A6 and CYP2B6 are known targets of ER α (Higashi et al., 2007; Lo et al., 2010; Koh et al., 2012).

Induction of CYP2C8 and CYP3A5 expression by progesterone is noteworthy. CYP2C8 and CYP3A5 are targets of PXR action, but PXR-mediated induction of the genes is known to be minor (Gerbal-Chaloin et al., 2001; Burk et al., 2004); the magnitude of CYP2C8 and CYP3A5 induction by a PXR activator (such as rifampin) is typically less than 10% of CYP3A4 induction (Gerbal-Chaloin et al., 2001; Rae et al., 2001). Interestingly, however, in our study, progesterone enhanced CYP2C8 and CYP3A5 expression by the magnitude comparable to CYP3A4, suggesting that regulatory mechanism(s) independent of PXR activation may be involved in CYP2C8 and CYP3A5 induction by progesterone. CYP2C8 is responsible for oxidative metabolism of up to 5% of drugs cleared by phase I processes, and its substrates include repaglinide and arachidonic acids (Totah and Rettie, 2005). On the other hand, CYP3A5 has substrate selectivity overlapping with CYP3A4 (e.g., midazolam) (Williams et al., 2002), suggesting that clinically observed increases in midazolam metabolism during pregnancy may be due in part to CYP3A5 induction by progesterone. Whether pregnancy influences pharmacokinetics of CYP2C8 substrates remains to be determined.

In summary, we comprehensively characterized the effects of estradiol and progesterone on expression of major hepatic CYPs using human hepatocytes. Estradiol enhances CYP2A6, CYP2B6, and CYP3A4 expression whereas progesterone induces CYP2A6 (minor), CYP2B6, CYP2C8, CYP3A4, and CYP3A5 expression. While molecular mechanisms underlying these hormonal effects on CYP expression are yet to be determined, our findings potentially provide a

basis for optimizing drug therapy in pregnant women by better understanding for altered drug metabolism during pregnancy.

Authorship Contribution

Participated in Research Design: Choi, Koh, Jeong

Conducted Experiments: Choi, Koh

Contributed New Reagents or analytic tools: None

Performed Data analysis: Choi, Jeong

Wrote or contributed to the writing of the manuscript: Choi, Koh, Jeong

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Footnotes

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Legend for Figures

Fig. 1. Elimination of estradiol and progesterone in a representative batch of human hepatocytes. Primary human hepatocytes were treated with estradiol (1 μ M, A), or progesterone (1 μ M, B; 10 μ M, C), and the media were sampled at various time points. The hormone concentrations were determined by LC/MS/MS.

Fig. 2. Effects of estradiol on mRNA expression levels of major CYPs. (A) Primary human hepatocytes from five different donors (n=3/batch) were treated with estradiol (1 μ M) or vehicle (ethanol) for 72 hr (with regular media change; see text for details). mRNA levels of CYP isoforms were measured by qRT-PCR. Results are expressed as relative expression in mRNA levels by estradiol relative to vehicle treatment. (B) A batch of human hepatocytes (HH093) was treated with estradiol (0.01-1 μ M) or vehicle (ethanol) for 72 hr (with regular media change; see text for details) (n=3). mRNA levels of CYP isoforms were measured by qRT-PCR. *, $p < 0.05$; **, $p < 0.01$ in comparison with vehicle-treated control.

Fig. 3. Comparison of the effects of estradiol and prototypical inducers on expression and activities of CYP2A6, CYP2B6, and CYP3A4. Human hepatocytes from different donors (n=3/batch) were treated with vehicle (ethanol), estradiol (1 μ M), CITCO (100 nM), or rifampin (10 μ M) for 72 hr. (A) mRNA expression levels of CYP2A6, CYP2B6 and CYP3A4 were determined by qRT-PCR. (B) CYP-isoform selective probe substrates [coumarin (100 μ M) for CYP2A6, S-mephenytoin (100 μ M) for CYP2B6, or midazolam (15 μ M) for CYP3A4] were added to media, and metabolite concentrations were measured by using LC/MS/MS. n.d., not determined. *, $p < 0.05$; **, $p < 0.01$ in comparison with vehicle-treated control.

Fig. 4. Increased CYP2C9 and CYP2E1 activities by estradiol. Human hepatocytes from different donors (n=3/batch) were treated with vehicle (ethanol; 0.1 %), estradiol (1 μ M), phenobarbital (0.5 mM), or ethanol (100 mM) for 72 hr. CYP-isoform selective probe substrates [(A) diclofenac (100 μ M) or tolbutamide (100 μ M); (B) *p*-nitrophenol (100 μ M) or chlorzoxazone (100 μ M)] were added to media. The metabolite concentrations in the media were measured by using LC/MS/MS. n.d., not determined. *, $p < 0.05$; **, $p < 0.01$ in comparison with vehicle-treated control.

Fig. 5. Effects of progesterone on mRNA expression levels of major CYPs. (A) Primary human hepatocytes from six different donors (n=3/batch) were treated with progesterone (10 μ M) or vehicle (ethanol) for 72 hr (with regular media change; see text for details). mRNA levels of CYP enzymes were measured by qRT-PCR. (B) A batch of human hepatocytes (HH101) were treated with progesterone (0.1-10 μ M) or vehicle (ethanol) for 72 hr with regular media change (n=3). mRNA levels of CYP isoforms were measured by qRT-PCR. *, $p < 0.05$; **, $p < 0.01$ in comparison with vehicle-treated control.

Fig. 6. Comparison of effects of progesterone and prototypical inducers on expression and activities of CYP2A6, CYP2B6, and CYP3A4. Human hepatocytes from three different donors (n=3/batch) were treated with vehicle (ethanol), progesterone (PRG, 10 μ M), CITCO (100 nM), or rifampin (10 μ M) for 72 hr. (A) mRNA expression levels of CYP2A6, CYP2B6, CYP2C8, CYP3A4, and CYP3A5 were determined by qRT-PCR. (B) CYP-isoform selective probe substrates [coumarin (100 μ M) for CYP2A6, S-mephenytoin (100 μ M) for CYP2B6, or midazolam (15 μ M) for CYP3A4] were added to media, and metabolite concentrations were measured by using LC/MS/MS. n.d., not determined. *, $p < 0.05$; **, $p < 0.01$ in comparison with vehicle-treated control.

Fig. 1

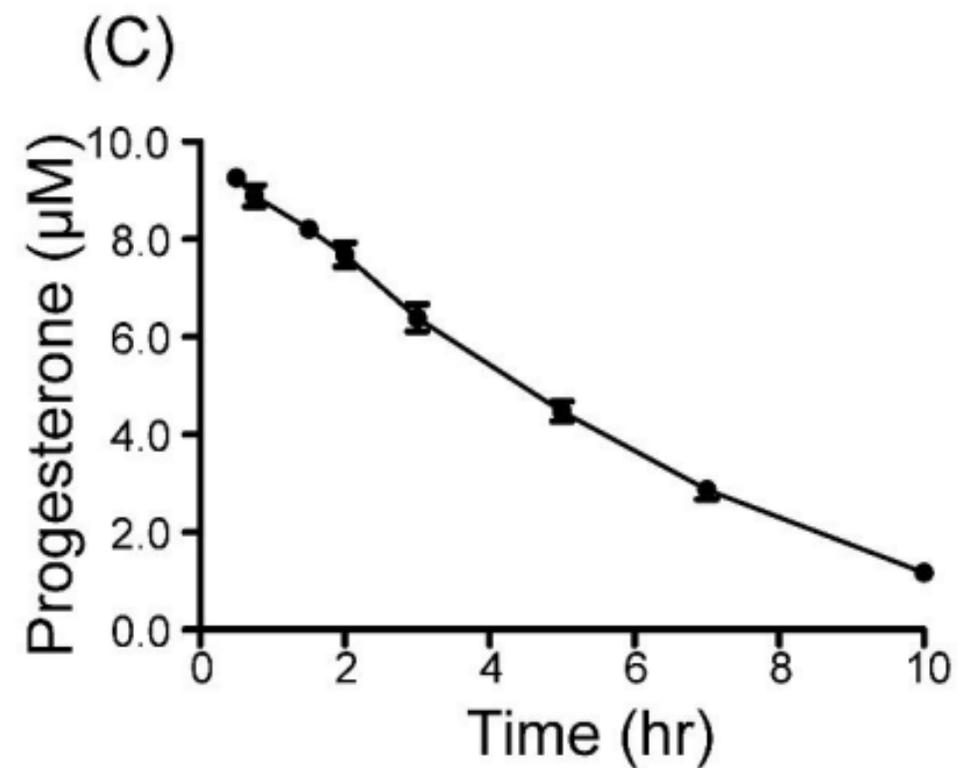
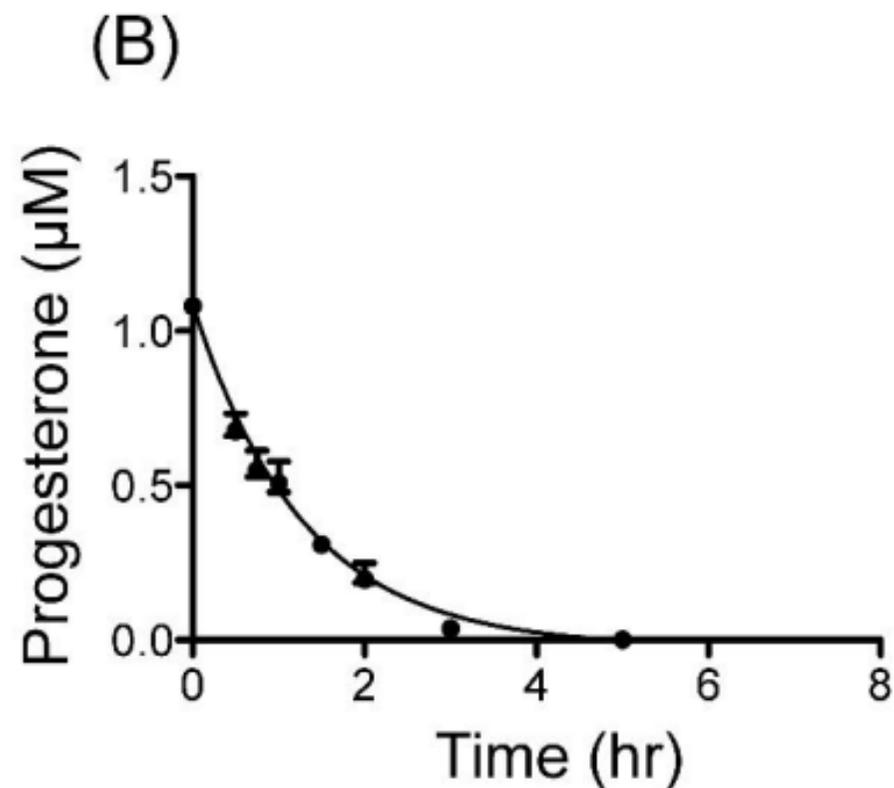
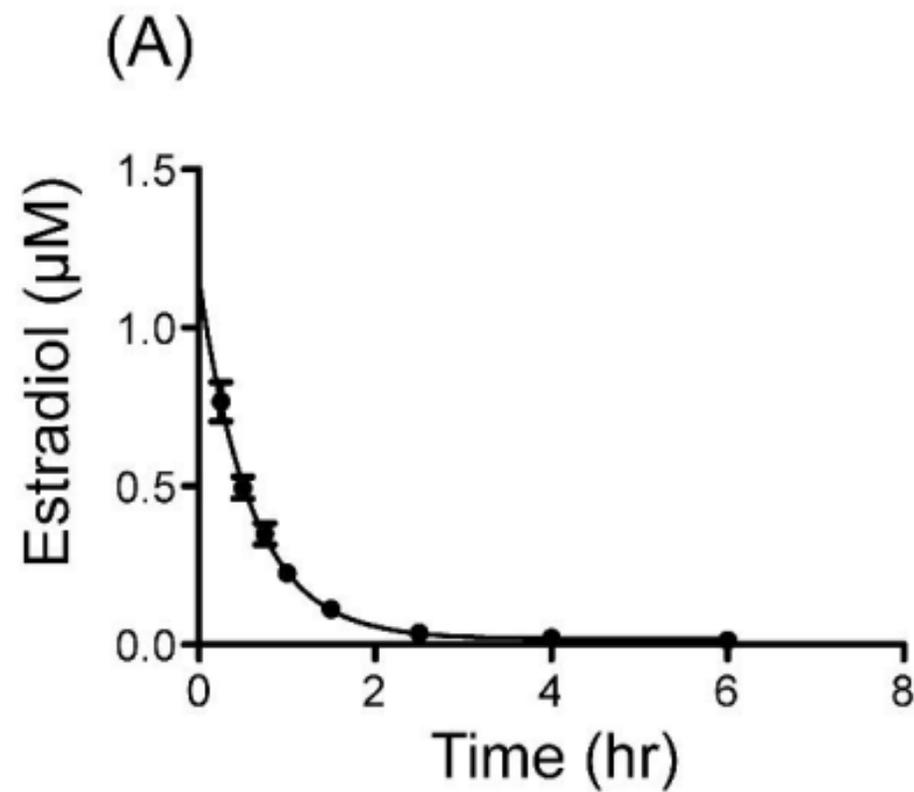
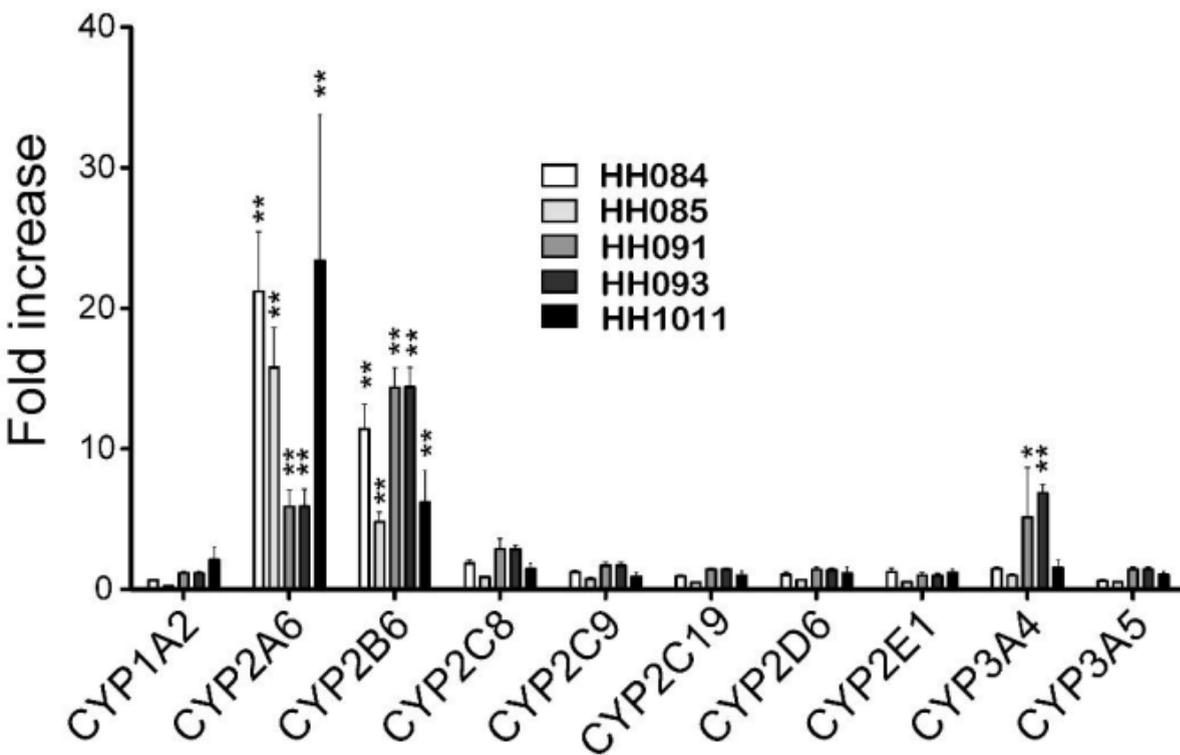


Fig. 2

(A)



(B)

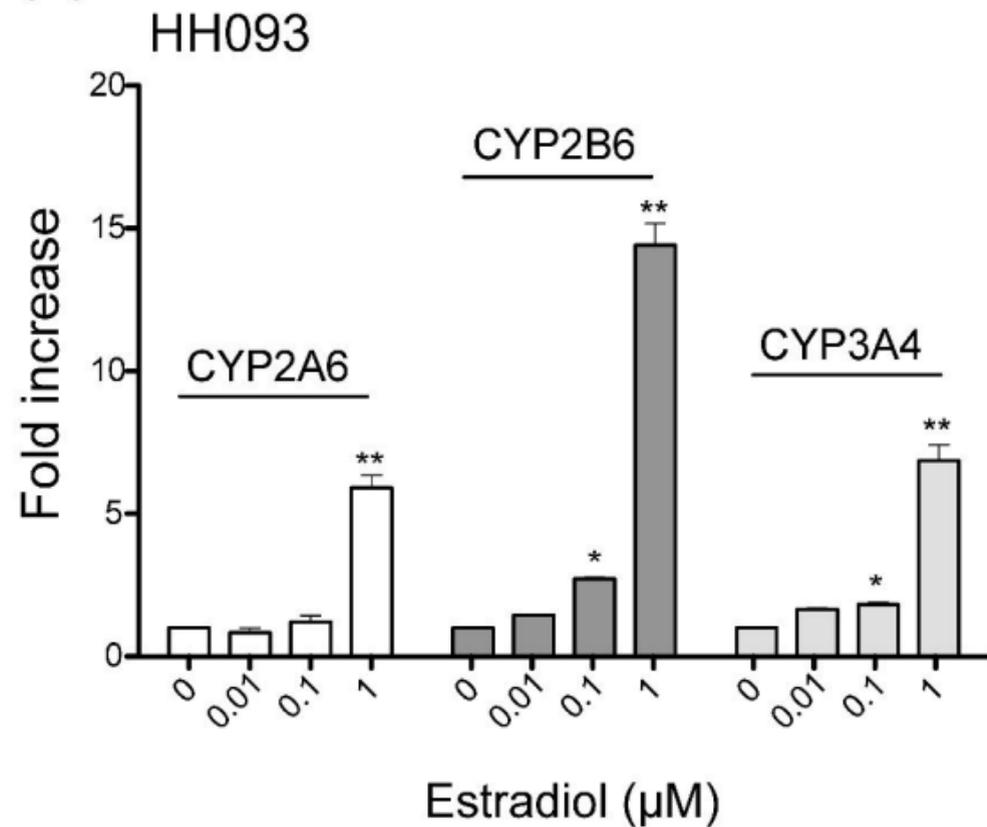
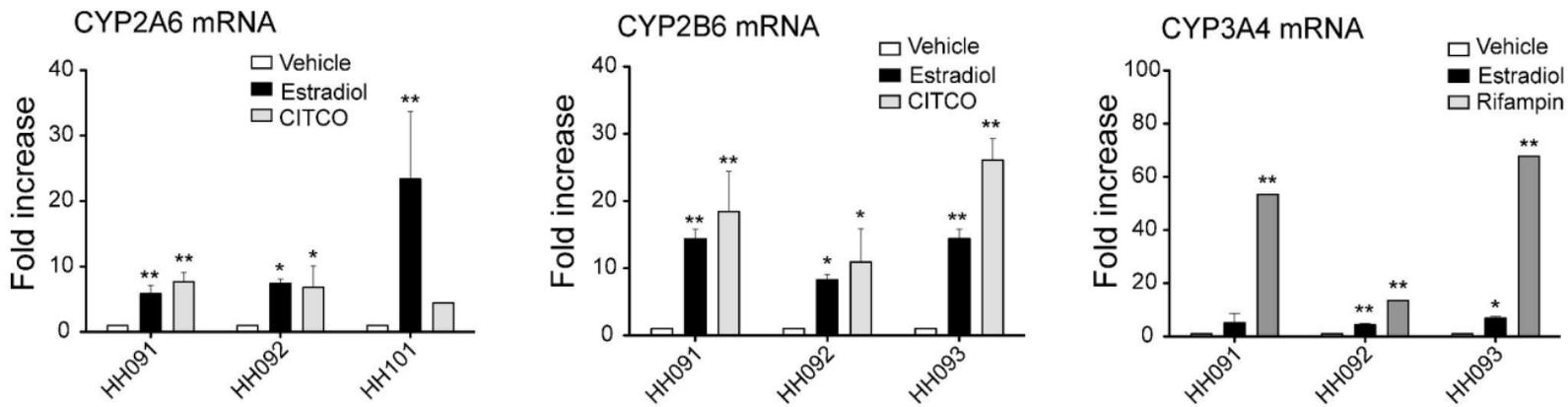


Fig. 3

(A)



(B)

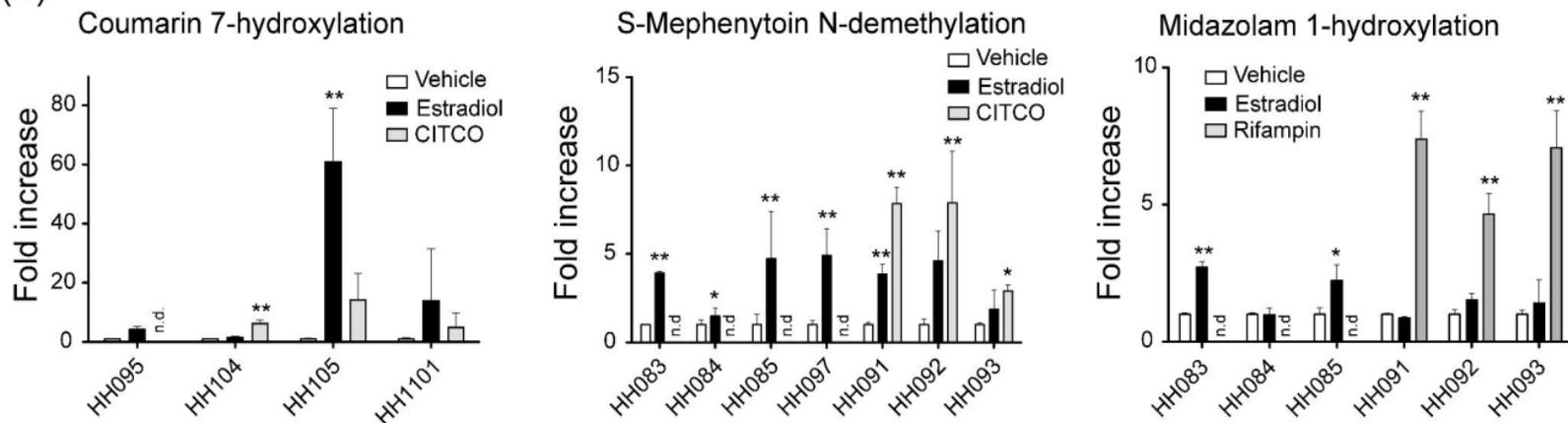
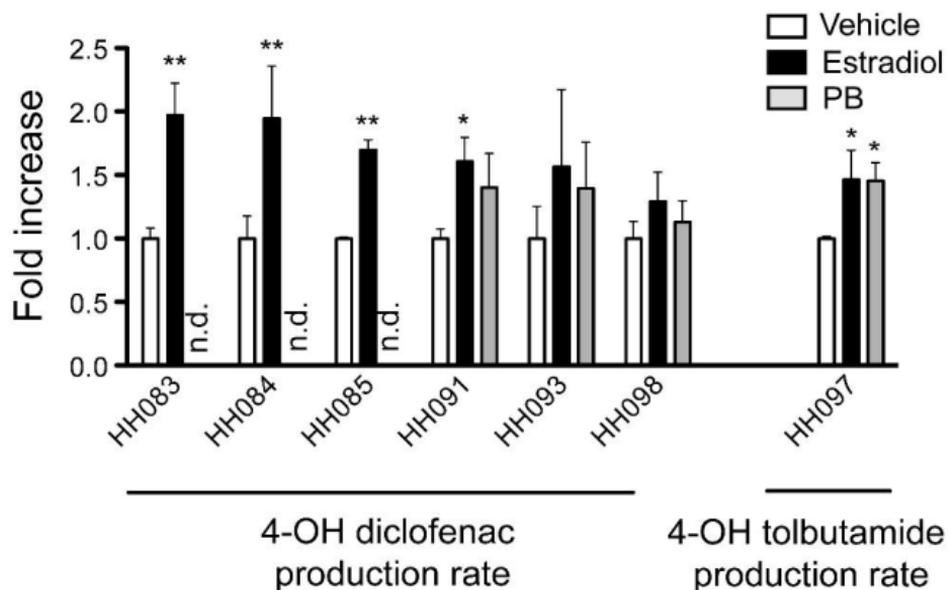


Fig.4

(A) CYP2C9



(B) CYP2E1

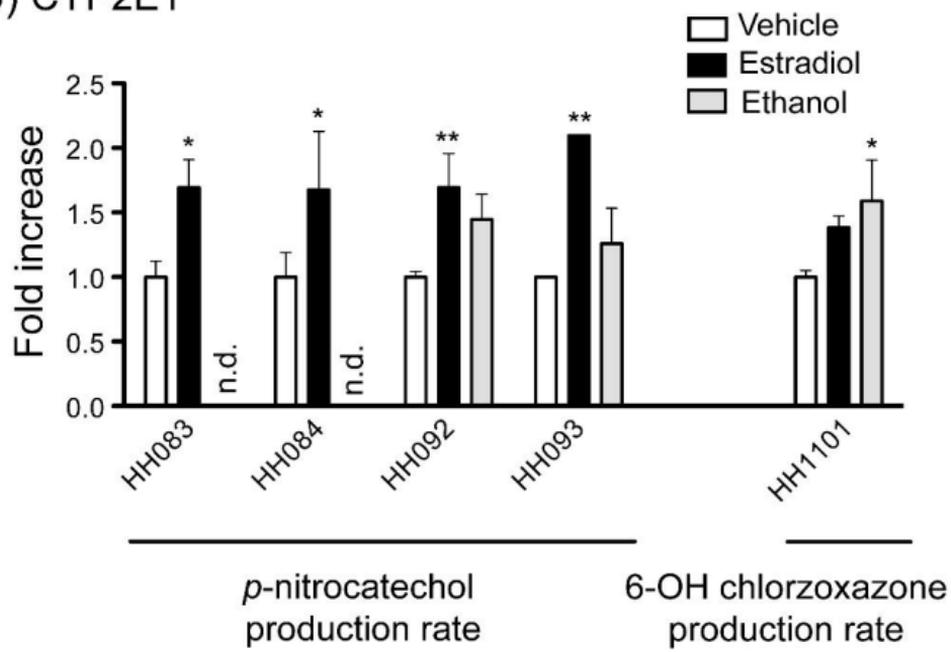
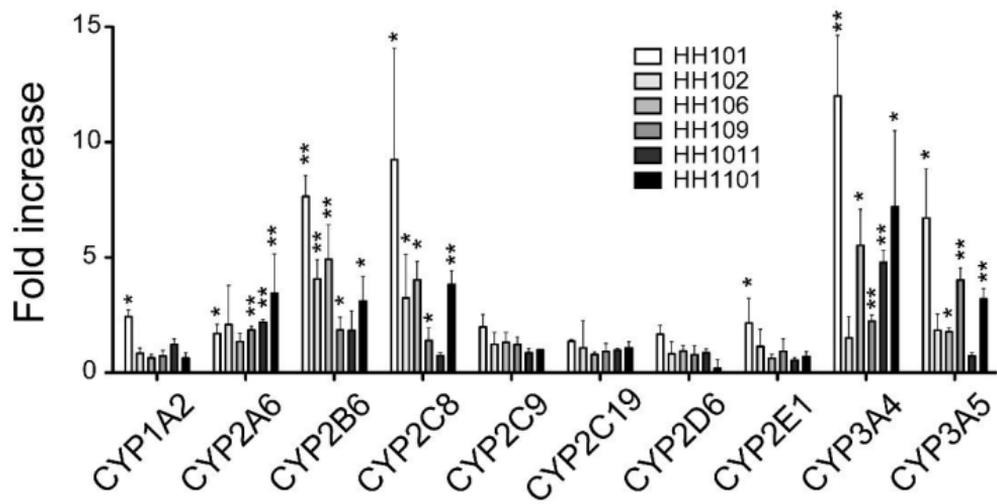


Fig. 5

(A)



(B)

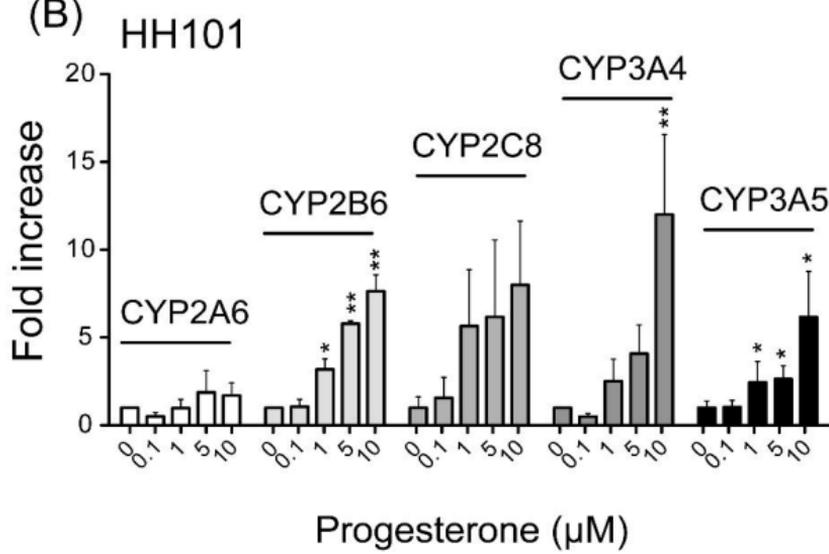
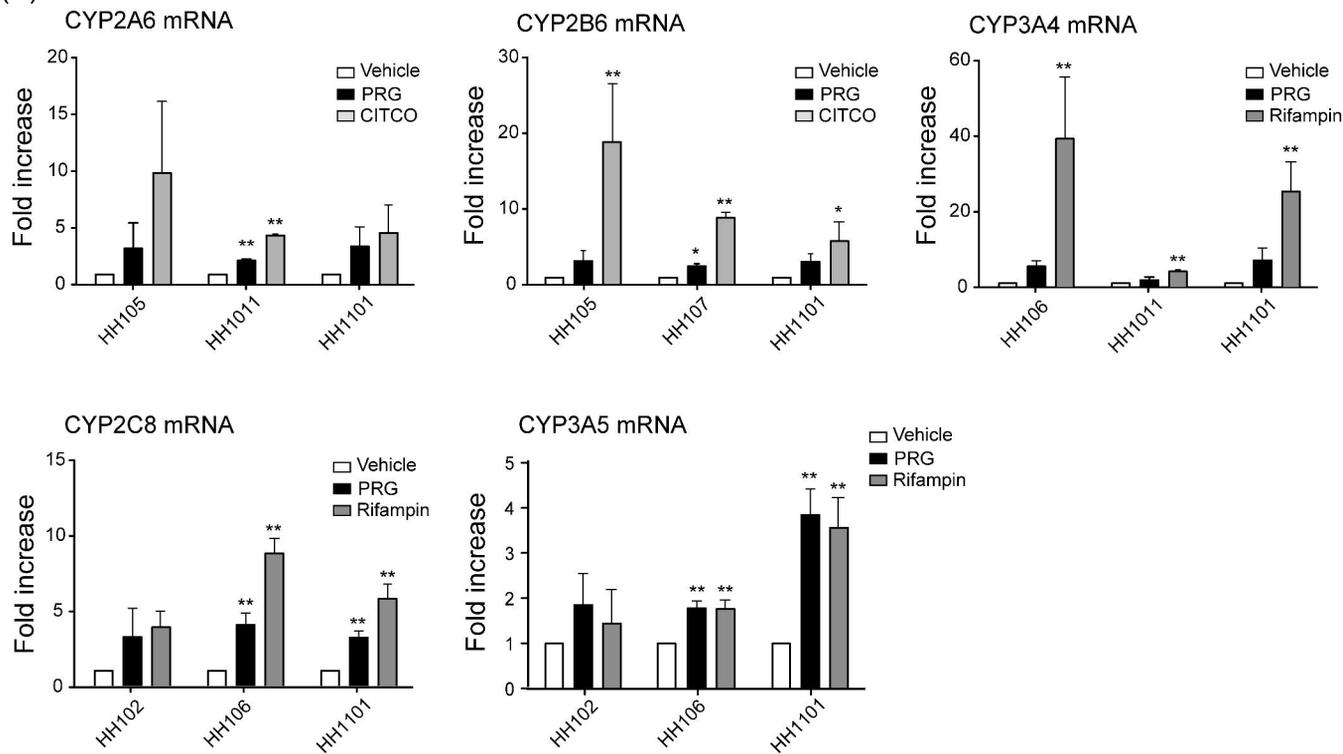


Fig. 6

(A)



(B)

