line. Placenta 24, 45-52. PMID: 12495659. Publisher's official version: <http://dx.doi.org/10.1053/plac.2002.0876>. Open Access version: http://kuscholarworks.ku.edu/dspace/.

Paper citation: Avery, M.L., Meek, C.E., and Audus, K.L. (2003) The presence of inducible cytochrome P450 types 1A1 and 1A2 in the BeWo cell line. Placenta 24, 45-52. PMID: 12495659

Abstract: The activity and inducibility of cytochrome P450 systems (CYP1A1:1A2) of the human placenta were assessed in a representative human trophoblast-like cell line, BeWo. The activity of CYP1A1 and CYP1A2 in microsome preparations from human liver, placenta, primary cultures of human cytotrophoblast, and BeWo cells was measured by *O*-dealkylation of 7-ethoxyresorufin (EROD) and 7-methoxyresorufin *O*-demethylation (MROD), respectively. Results indicated high EROD and MROD activity associated with human liver microsomes, sometimes comparable activities in human placenta microsomes prepared from smokers, and relatively low activities in human placenta microsomes from nonsmokers and in the primary cultures of cytotrophoblasts isolated from nonsmokers. Microsomes from BeWo cell monolayers exhibited the lowest EROD and MROD activities relative to all other microsome preparations. However, compared to primary cultures of normal trophoblasts, the EROD activity of the BeWo cells was far more sensitive to typical inducers, 3-methylcholanthrene, 1,2-benzanthracene, and β-naphthoflavone. EROD activity in BeWo cells was induced approximately 200-fold by 3-methylcholanthrene. Both EROD and MROD activity in BeWo cells was readily induced by 1,2-benzanthracene, 100-fold and 60-fold, respectively. After induction with 1,2-benzanthracene, the CYP1A1 selective inhibitor, α-naphthoflavone, and the CYP1A2 selective inhibitor, furafylline, effectively inhibited enzyme activities with IC_{50}'s of 2.4μM and 12.8μM, respectively, in microsomes from both trophoblasts culture systems. These results show that major cytochrome P450 forms present in human placenta are present and inducible in BeWo cells, a potential model for investigation of drug metabolism mechanisms in the human trophoblast.

Text of paper:

The Presence of Inducible Cytochrome P450 Types 1A1 and 1A2 in the BeWo Cell Line

M.L. Avery, C.E. Meek, and K.L. Audus

Department of Pharmaceutical Chemistry, School of Pharmacy, The University of Kansas, Lawrence Kansas 66047.

Avery, M.L., Meek, C.E., and Audus, K.L. (2003) The presence of inducible cytochrome P450 types 1A1 and 1A2 in the BeWo cell line. Placenta 24, 45-52. PMID: 12495659. Publisher's official version: <http://dx.doi.org/10.1053/plac.2002.0876>. Open Access version: http://kuscholarworks.ku.edu/dspace/.

INTRODUCTION

Cytochrome P450 systems in the human placenta are induced by polycyclic hydrocarbons present in cigarette smoke resulting in altered steroid or xenobiotic metabolism (Pasanen et al., 1990; Pasanen and Pelkonen, 1990; 1994; Lee and Riddick, 2000). Polycyclic aromatic hydrocarbons are substrates for cytochrome P450 form 1A1 (CYP1A1) and exposure to these molecules correlates with low birth weight in man and in other species (Khera el al., 1972; Shum et al., 1979; Allen and Barsotti, 1976). The CYP1A subfamily is primarily extrahepatic and includes types 1A1 and 1A2 which are about 70% homologous in amino-acid sequences (Nerurkar et al., 1993).

Human CYP1A1 and CYP1A2 forms are under the regulation of the *Ah* locus and retain activity toward polycyclic aromatic hydrocarbons. The CYP1A2 form preferentially retains activity toward aromatic and heterocyclic amines (Butler et al., 1989). CYP1A1 is the more persistent of several P450 forms present throughout pregnancy (Hakkola et al., 1996a; 1996b). By contrast, CYP1A2 has a relatively minor constitutive expression in the human placenta (Pasanen and Pelkonen, 1994). Considering the role of cytochrome P450 in drug metabolism, there is a need to develop models to elucidate the significance of drug interactions with human placenta enzymes in situations where the mother is exposed to tobacco smoking and other environmental conditions (Pasanen and Pelkonen, 1994; Zevin and Benowitz, 1999).

The objective of this study was to characterize the activity and inducibility of selected cytochrome P450 systems, CYP1A1:1A2, in microsomes from representative *in vitro* trophoblast systems including a human trophoblast-like cell line, BeWo. The presence of inducible CYP1A1 and CYP1A2 forms in appropriate *in vitro* systems would provide useful tools to study environmental factors, drugs, drugs of abuse effects on the metabolism in the human trophoblast. Cytochrome P450 activity in the microsomes prepared from the placenta of smokers, the placenta of nonsmokers, primary cultures of

cytotrophoblasts isolated from the placenta of nonsmokers, and the BeWo cells was measured by *O*-dealkylation of specific substrates for CYP1A1, 7-ethoxyresorufin, and CYP1A2, methoxyresorufin (Dutton and Parkinson, 1989). To verify inducibility of metabolism, BeWo cell monolayers and primary cultures of human cytotrophoblasts were exposed to CYP1A1:A2 inducers, 3-methylcholanthrene, 1,2-benzanthracene, and β-naphthoflavone. We also explored the effects of a specific CYP1A1 inhibitor, α-naphthoflavone, on 7-ethoxyresorufin *O*-dealkylation (EROD), and a specific mechanism-based CYP1A2 inhibitor, furafylline, on 7-methoxyresorufin *O*-demethylation (MROD) in the BeWo cell system.

MATERIALS AND METHODS

BeWo cell culture.

The BeWo cell line was originally derived from a human choriocarcinoma (Pattillo and Gey, 1968; Pattillo et al., 1979). The BeWo (b30) was obtained from Dr. Alan Schwartz (Washington University, St. Louis, MO) and grown in culture as detailed in earlier work (Liu et al., 1997; Kenagy et al., 1998). Briefly, BeWo cells were continuously cultured in DMEM with 10% heat-inactivated FBS containing 0.37% sodium bicarbonate and 1% antibiotics (10,000 U/ml penicillin and 10 mg/ml streptomycin). The cells were routinely maintained in 150 cm Corning flasks at pH 7.4 under 5% CO_2 and 95% humidity at 37°C. The cells were harvested by brief exposure (2-3 minutes) to a trypsin EDTA solution (0.25% trypsin and 0.02% EDTA in PBS). The culture medium was exchanged every 48 hours. Consistency in exponential growth required 1:10 split for each passage. The passages used for the following experiments were 22-38.

Human cytotrophoblast cell culture

The study protocol and use of human placentas in this work was approved by the University of Kansas Advisory Committee on Human Experimentation (ACHE#8904).

Term placentas were received from unremarkable pregnancies of anonymous volunteers who indicated that they were either nonsmokers or smoked on a regular basis. Except for placentas used for cell isolation and primary cell culture described below, placentas were washed with ice-cold normal saline and immediately frozen at –70°C after delivery. For each preparation, placenta tissue was sampled from three to four delivered term placentas from mothers who indicated either that they were non-smokers or smoked cigarettes on a regular basis. For preparation of primary cultures, purified human cytotrophoblasts were isolated by the methods of enzymatic digestion and Percoll gradient centrifugation (Kenagy et al., 1998). The partially purified cytotrophoblasts were collected washed once with DMEM and resuspended with plating media consisting of DMEM with 10% fetal bovine serum. 0.1% mg/ml L-glutamine and 50 μg/ml gentamicin.

Microsome preparation

Microsomal fractions from BeWo cells were prepared according to Hakkola et al. (1996a). Briefly, the cells were grown in 150-cm flasks until confluent monolayers were formed. The cells were rinsed with phosphate-buffered saline, pH 7.4, and bathed in a buffer containing 50mM Tris-HCL, pH 7.4, 150 mM KCl, and 2 mM EDTA. The cells were removed from the flasks and homogenized with a glass homogenizer in a 1:4 vol of 0.1 M sodium potassium phosphate buffer (pH 7.4) containing 20% (v/v) glycerol. Cell extracts were centrifuged at 1000g for 10 minutes and the supernatant was centrifuged at 10,000g for 30 minutes. The resulting supernatant was centrifuged at 100,000g for 60 minutes. The supernatant from the last spin was discarded and the microsomal pellet was suspended in 0.25 M sucrose and stored at –70°C.

Western blots

Microsomes prepared from BeWo cells, cytrophoblasts, and whole placental tissue from smokers and nonsmokers (0.5 μg protein) and were subjected to SDS-PAGE, 12% acrylamide Tris-glycine. Microsomal proteins were transferred electrophoretically to polyvinylidene difluoride (PVDF) membranes for 6 hours with a 35mA current. A 10% nonfat dry milk and 2% bovine serum albumin solution in 10mM Tris-HCl, pH 7.4, was used to block nonspecific binding. Microsomal P450 was detected by CYP1A1/CYP1A2 (10 μg/ml) polyclonal antibody (Xentotech, Kansas City, KS) and CYP1A2 (10μg/ml) monoclonal antibody (Gentest, Woburn, MA). Antibody solutions were prepared in 10 mM Tris-HCL, pH 7.4, containing 0.05% Tween 20. Secondary antibody, anti-mouse IgG (Fab) peroxidase conjugate was prepared 1:80,000 fold in 10 mM Tris-HCl containing 0.05% Tween 20 and incubated with PVDF membrane for 1 hr at 25°C. CYP1A1 and CYP1A2 were detected by enhanced chemiluminescence by incubating PVDF immunoblots at 25°C for 1 minute and exposure to Hyperfilm-HCL.

Enzyme assays (EROD and MROD)

Microsomal cytochrome P450 activity was measured fluorometrically by *O*-dealkylation of 7-ethoxyresorufin (EROD) and *O*-demethylation of 7-methoxyresorufin (MROD) producing resorufin (Dutton and Parkinson, 1989). Reaction mixtures of 1 ml final volume were incubated at 37°C. The reaction mixture contained potassium phosphate buffer (100mM, pH 7.4), MgCl (3 mM), EDTA (1 mM), NADPH (1.0 mM), glucose 6-phosphate (5.0 mM), glucose 6-phosphate dehydrogenase (1 unit/ml) and 7-ethoxyresorufin/ methoxyresorufin (2.5 mM in DMSO). Blanks did not contain NADPH. Reactions were initiated by adding NADPH and stopped after 8 minutes with 2 ml acetone. Cellular protein was removed by centrifugation at 1000g for 10 minutes and the supernatant transferred to cuvettes.

Fluorescence was measured at an emission of 590 nm and the excitation at 530 nm in an SLM-Aminco 4800 spectrofluorometer.

***In vitro* Induction of CYP1A1:1A2**

7-Alkoxyresorufin *O*-dealkylase and 7-Methoxyresorufin *O*-demethylation activities were measured by the fluorometric method of Burke and Mayer with modifications (Burke et al., 1978; 1985). BeWo cells were seeded at a density of 1.6 x 10^5 cells/cm^2 in six well-plates (Nunclon). Experiments were conducted when the cells formed confluent monolayers and all experiments were conducted in culture medium. The cells were washed three times with PBS and treated with 10 μM dicumerol [3,3'-methylene-bis(4-hydroxycoumarin)] and 8 μM of either 7-ethoxyresorufin or 7-methoxyresorufin. The cells were incubated at 37°C for 30 minutes. After the incubation, a 750-μl aliquot of culture medium from each well was removed and placed in a glass test tube and 250 μl of a β-glucuronidase (15 Fishman U)/arylsulfatase (120 Roy U) solution in 0.1 M sodium acetate (pH 4.5) was added to each test tube. The test tubes were incubated for 2 hours at 37C. A 2.0-ml aliquot of 95% ethanol was then added to each test tube and the samples were centrifuged for 10 minutes at 3000 rpm. A 2-ml sample of the supernatant was placed in a cuvet for fluorescence detection (SLM fluorometer, excitation 530 nm, emission 590 nm). The amount of resorufin formed in the reaction was determined based on a resorufin standard curve and corrected for the volume in the original sample. A BCA protein assay (Pierce) was conducted on the monolayers in order to calculate the amount of resorufin formed per milligram protein. To measure 1,2-benzanthracene, 3-methylcholanthrene, and β-napthoflavone induction of CYP1A1:1A2 BeWo cells were treated 24 and 48 hours prior to assaying EROD and MROD. Inhibitory effects of α–naphthoflavone (7,8-benzoflavone) on CYP1A1 and furafylline on CYP1A2 were studied in BeWo cells after 24 hours exposure to 100μM of 1,2-benzanthracene.

Statistical analysis

An ANOVA followed by a Tukey's multiple comparisons test was applied to the data in this study. Where appropriate, statistical differences were indicated by a *P*-value of less than 0.05. Data are expressed as mean ± standard error of the mean (SEM).

RESULTS

Microsomes were prepared from liver, placenta from mothers who indicated they smoked cigarettes on a regular basis, placenta from nonsmokers, primary cultures of human cytotrophoblasts isolated from the placenta of nonsmokers, and BeWo cell monolayers. The microsomes were assayed for EROD and MROD activity based on resorufin formation and results are summarized in Table 1. The liver exhibited the greater EROD and MROD activities in microsomes as expected, however, activities in the placentas from smokers approached the liver at least with respect to EROD activity in some instances. Samples were collected from multiple placentas and two pools were created to show the variability in induction that was observed in the placentas from smokers. EROD and MROD activities in microsomes prepared from the placentas of nonsmokers were consistently low. Microsomes prepared from human cytotrophoblasts isolated from placentas of nonsmokers grown in primary culture also retained low EROD activity comparable to the levels observed in samples from placental tissue samples of nonsmokers. The BeWo cells exhibited consistently low EROD and MROD activity overall.

In primary culture, isolated human cytotrophoblasts from the placentas of nonsmokers were shown to retain the low levels of EROD activity as indicated in Table 1. In these studies, EROD activity in the primary cultures was assayed after 8 days in culture with monolayers that were approximately 60% confluent. As illustrated in Figure 1, EROD activity was modestly altered in these primary cell cultures by

the inducers 3-methylcholanthrene and β-naphthoflavone. Both inducers produced increases in EROD maximally of about 2-fold.

Based on the modest inducibility of the primary cultures, further experiments were carried out with the BeWo cell line which showed a greater response to the individual inducers. The BeWo cells exhibited basal EROD and MROD activity that was lower than the primary cultures of human cytrophoblasts and activity observed in microsomes from placentas of nonsmokers (Table 1). By contrast, however, BeWo cells were far more responsive to selected inducers than the primary cultures of human cytotrophoblasts. 3-methylchloranthrene, β-naphthoflavone, and 1,2-benzanthracene induced EROD and MROD activity in BeWo cells in a dose-dependent manner following a 24 hour treatment. Induction of EROD by 3-methylcholanthrene was concentration-dependent and nearly 200-fold over the control activity as also presented in Figure 1. The EC_{50} for 3-methylcholanthrene induction was 5.2 μM as calculated by nonlinear regression. β-naphthoflavone induction of EROD activity in BeWo cells is summarized in Figure 2. At higher concentrations of the inducer, a maximal induction of about 20-fold was observed with β-naphthoflavone alone and approximately 50-fold when applied in combination with dexamethasone.

Immunoblotting experiments shown in Figure 3A, indicate CYP1A1 and CYP1A2 immunoreactivity from microsomes isolated from primary cultures of cytotrophoblasts (lane 3) compared to microsomes from whole placental tissue of nonsmokers (lane 1), microsomes from whole placental tissue samples from smokers (lane 2) and microsomes from rat liver as a positive control (lane 4). Blots for microsomal protein from the primary cultures were qualitatively similar to the whole placental tissue sample for nonsmokers. Confirmation of inducible CYP1A1:1A2 in cytotrophoblasts by was observed in microsomal protein obtained from cells harvested 8 days post seeding *in vitro* and is illustrated in Figure 3B. CYP1A1 and CYP1A2 immunoreactivity from cytotrophoblast microsomes of

primary cultures from nonsmokers (lane 1) was visibly enhanced relative to the immunoreactivity from cytotrophoblast microsomes of primary cultures microsomes from whole placenta of nonsmokers (lane 3) in Figure 3A, and microsomes from whole placenta of maternal smokers (lane 1) in Figure 3A. The immunoreactivity in the positive controls, microsomes from whole placenta of smokers (lane 2), microsomes form rat liver-induced with 3-methylcholanthrene (lane 3) and non-induced human liver (lane 4) were relatively strong as illustrated in Figure 3B.

To verify induction of CYP1A1:1A2 in BeWo cells, the cells were to exposed to chemical inducers for 72 hours. In Figure 4, relative to 3-methylcholanthrene induced rat liver microsomes (lane 1), uninduced BeWo microsomes (lane 2) are shown alongside protein from microsomes from cells induced with 10μM of 3-methylchloranthrene (lane 3), or 10μM of β-naphthoflavone (lane 4), or 10μM of 1,2-benzanthracene (lane 5). Immunoreactive CYP1A1 was observed to be inducible to a significant degree by two of the three compounds tested with optimal response by 3-methylchloranthrene and a lesser response by 1,2-benzanthracene.

Figure 5 shows preferential 1,2-benzanthracene induction on EROD activity compared to MROD activity in the BeWo cells. At higher concentrations, 1,2-benzanthracene induced EROD and MROD activity by about 100- and 60- fold, respectively over control levels. Phenobarbital a classic inducer of CYP2B1 has been shown to increase 7-pentoxyresorufin *O*-dealkylase (PROD; a substrate for CYP2B1) but not EROD activity in brain microsomes (Dhawan et al., 1999). In BeWo cells induction by a 24 hour exposure to 100 μM phenobarbital increased MROD activity about 30-fold (12.7 $\pm$ 1.0 pmol/mg/min) compared with a more modest 2.5-fold stimulation of PROD (1.0 $\pm$ 0.3 pmol/ml/min). Induction of EROD by phenobarbital was not observed in BeWo cells (data not shown).

To establish that the induced EROD and MROD activities were specific to CYP1A1 and CYP1A2, we looked at the effects of specific inhibitors, 7,8-benzoflavone (or α-naphthoflavone) and furafylline,

respectively, following induction by 100 μM 1,2-benzanthracene for 24 hours. Results summarized in Figure 6 shows 7,8-benzoflavone effectively inhibited EROD activity, IC_{50} = 2.4 μM, and furafylline inhibits MROD activity, IC_{50} = 12.8 μM, respectively.

DISCUSSION

The enzyme activities of 7-ethoxyresorufin *0*-dealkylase and 7-methoxyresorufin *0*-demethylation were measured to study induction and inhibition mechanisms of CYP1A1 and CYP1A2, respectively in placental tissues and trophoblast culture systems. It has been reported that EROD is represented by CYP1A1 and CYP1A2 (Eugster et al., 1993; Shimada et al., 1997; Nakajima et al., 1999). Several reports suggest MROD is a suitable marker of CYP1A2 (Nerurkar et al., 1993; Iba et al, 1999; Sakuma et al., 1999).

Ex vivo placenta microsomes were compared in two parallel studies and were based on the predetermined indication of regular maternal smoking without knowing the number or duration of cigarette smoking. Observed EROD and MROD levels measured in two pooled microme samples, each sample representing three to four different placentas. We observed significant differences in EROD and MROD activities among maternal smokers and nonsmokers. These studies suggested that inducible CYP1A1 and CYP1A2 are present in the human placentas surveyed here. Our results were consistent with previous reports that showed EROD and aryl hydrocarbon hydroxylase levels up to a 100-fold greater in placenta exposed to maternal smoking. Moreover, the magnitude of the activities found here as well as the variability of different sample pools from placenta was consistent with earlier studies reported for both smokers and nonsmokers (Pasanen et al., 1988; 1990; Hakkola et al., 1996a; Lee and

Riddick et al., 2000). These observations for the basis for identifying cell culture systems that retain a similar expression and activity of major enzymes as well as the sensitivity to known chemical inducers.

In cytotrophoblasts isolated from term placenta and grown in primary culture, inducible CYP1A1 was increased by less than 2-fold by 3-methylcholanthrene and only 2- to 2.4-fold by β-naphthoflavone. These results may be due to absence of confluency of primary cells, the percentage of cells that adhere to cell culture plates, and or simply the magnitude of inducible P-450 enzymes *in vitro* (Madan et al., 1999). As a result of the modest responsiveness of the primary cultures, we pursued studies with the BeWo cell line which responded impressively to known chemical inducers. A 200-fold induction of CYP1A1 by 3-methylcholanthrene in BeWo cells was observed and the effect was concentration dependent with an estimated EC_{50} of ~5.16 μM. Studies with BeWo cells also indicated that dexamethasone increased the induction produced with β-naphthoflavone treatments. Previously, transcriptional regulation of AhR-mediated pathway induction with dexamethasone has been reported (Mathis et al., 1986; Prough et al., 1996). Results in that study supported the idea that CYP1A1 was induced by aryl hydrocarbons via a AhR-mediated pathway. In our study, we report a 51-fold increase in EROD after induction with dexamethasone and β-naphthoflavone compared with a 24-fold increase with β-naphthoflavone. Accordingly, our results support the possible role of the AhR-mediated pathway in the induction of BeWo cell EROD.

Northern blot analysis using mouse CYP1A1 cDNA has confirmed rat hepatic CYP1A1 (~2.7 Kb) and CYP1A2 (~2.0 Kb) induction after 6 hours post-dose with 3-methylcholanthrene (Lee and Riddick, 2000). In time course studies, a single intrapertioneal dose of 3-methylcholanthrene (40mg/Kg) in rodents also resulted in a 179-fold increase in CYP1A transcription after 6 hours (Lee and Riddick, 2000). These results were also supported by findings that transcriptional rates of CYP1A1 and CYP1A2 increased by 3-hours and were maximal 12 hours after 3-methylcholanthrene exposure (Lee and Riddick, 2000). In

BeWo cells, inducible CYP1A1:1A2 was maximal after 24 hours induction with 3-methylcholanthrene. Apparent cell degradation was observed post 24 hours with 3- methylcholanthrene (data not shown). A decrease in EROD and MROD levels were observed in BeWo cells after a 24 hours exposure to 1,2-benzanthracene. Consequently, the time course for maximal *in vitro* induction was 24 hours or less and was apparently somewhat slower than the time course for maximal induction in rodents.

Immunoblots confirmed CYP1A1:1A2 induction in placentas obtained from smokers. Our results were in agreement with immunoblots from induced microsomes from rodent liver exposed to dose-dependent nicotine feeding (Iba et al., 1999). The immunoblots confirmed retention of a modest induction of enzyme expression in primary cultures of cytotrophoblasts from nonsmokers following treatment with 10 μM of 3-methylcholanthrene. Additionally, immunoblotting confirmed induction by 3-methylcholanthrene in microsomes isolated from BeWo cells. Induction of BeWo cells with β-napthoflavone and 1,2-benzanthracene also increased immunoreactive CYP1A1, however less than observed with 3-methylcholanthrene. The qualitative observations of the immunoblots for CYP1A1:1A2 are in general agreement with the biochemical data in this study.

Many P450 chemical inhibitors are well characterized in primary cell cultures of hepatocytes (Pichard et al., 1990). To assess the contributions of CYP1A1 and CYP1A2 to EROD and MROD measures in BeWo cells, we used to known inhibitors following exposure to 1,2-benzanthracene. In BeWo cells, effective inhibition of CYP1A1 by 7,8-benzoflavone and CYP1A2 by furafylline was observed 24 hours after induction with 1,2-benzanthracene. Results show that 7,8-benzoflavone inhibited 7-ethoxyresorufin *O*-deethylase by approximately 80% with an apparent IC_{50} of 2.4 μM. Furafylline, a mechanism-based inhibitor specific for CYP1A2, inhibited methoxyresorufin *O*- demethylation by greater than 70% with an observed IC_{50} of 12.8 μM. Previously, Sesardic et al. (1990a;1990b), reported furafylline inhibited human placenta phenacetin *O*-deethylase with an IC_{50} of 46μM. Therefore,

considering the extent of inhibition together with the IC_{50} values suggest that 7,8-benzoflavone is a strong inhibitor of CYP1A1 enzymatic activity and furafylline is a moderate inhibitor of CYP1A2 and MROD. Our data would suggest that BeWo cells may be a suitable matrix to screen compounds using competitive and mechanism based inhibitors of these enzymes although additional studies are required to further validate our observations.

Hakkola et al. (1996b) detected CYP1A1:A2 mRNA during the first trimester without significant CYP1A2 mRNA at term. Results reported herein suggest inducible CYP1A1 levels in cytotrophoblasts from term placenta and BeWo cells consistent with that work. By contrast, our studies confirm CYP1A2 protein and enzymatic activity from placenta based on immunoblotting and inducible CYP1A2 demethylation. In BeWo cells, induction of CYP1A2 with phenobarbital increased MROD 30-fold. Induction studies with phenobarbital exposure in rodent livers have been shown to have immunoreactivities with CYP1A1:A2 antibodies (Sakuma, et al., 1999). In other rodent studies, phenobarbital induced CYP1A2 and further demonstrated in rodent primary hepatocytes that CYP1A2 transcription and enzyme levels are inducible with exposure to phenobarbital (Nemoto and Sakurai, 1995; Sakuma et al., 1999). Moreover, CYP1A2 has been subsequently suggested to be a member of the phenobarbital-inducible genes in mice and that the AhR-mediated pathway does not play a role in the induction (Sakuma et al., 1999). Similarly, In a recent communication, CYP1A2 induction was demonstrated in Hepa-1 cells, a hepatocyte cell line (Sindhu et al., 2000). In HEPG2 cells, another hepatocyte cell line, CYP1A1 was detectable after induction by polycyclic aromatic hydrocarbons (PAH), however, CYP1A2 was not detectable after PAH induction (Vakharia et al., 2001). Thus, in liver CYP1A2 expression has been extensively studied *in vivo*, *ex vivo*, and *in vitro* and other reports describe the presence of CYP1A2 in other tissues including the brain and with cultured umbilical vein endothelium (Farin and Omiecinski, 1993; Farin et al., 1994; McDonnell et al., 1992; Sindhu et al., 2000). It is not surprising that CYP1A2 is inducible in a placental cell line. The degree to which CYP1A2 is expressed and

functional in a given tissue such as the placenta at term may depend on the type and prior chemical exposures as previously proposed by Pasanen and Pelkonen (1994).

In summary, comparisons shown with primary cytotrophoblasts and placenta microsomes from maternal smoking that inducible CYP1A1/A2 activities are present at term. BeWo cells retain low CYP1A1/A2 activity that is consistent with the placenta of nonsmokers. However, data reported in these studies show CYP1A1/A2 are readily inducible in BeWo cells and implies that this system may be a good model for studying CYP1A1/A2 induction in the human trophoblast.

ACKNOWLEDGEMENTS

This work was supported by a grant from National Institute of Drug Abuse (NIDA N01DA-4-7405). The authors would also like to acknowledge the support of Corning Costar Corporation for support of the Cellular and Molecular Biopharmaceutics Handling Laboratory.

REFERENCES

Allen, J.R. & Baarsotti, D.A. (1976) The effects of transplacental and mammary movement of PCBs on infant rhesus monkeys. *Toxicology,* 6, 331-340.

Burke, M.D., Prough, R.A. & Mayer, R.T. (1978) Characteristics of a microsomal cytochrome P-448-mediated reaction ethoxyresorufin *O*-deethylation. *Drug Metabolism and Disposition,* 5, 1-8.

Burke, M.D., Thompson, S., Elcombe, C.R., Halpert, J., Haaparanta, T., & Mayer, R.T. (1985) Ethoxy-, pentoxy-and benzylphenoxazones and homologues: a series substrates to distinguish between different induced cytochromes P-450. *Biochemical Pharmacology,* 34, 3337-3345.

Butler, M.A., Iwasaki, M., Guengerich, F.P., & Kadlubar, F.F. (1989) Human cytochrome P-450pa (P-4501A2), the phenacetin O-deethylase, is primary responsible for the hepatic 3-demethylation of caffeine and N-oxidation of carcinogenic arylamines. *Proceedings of the National Academy of Science USA,* 86, 7696-7700.

Dhawan, A., Parmar, D., & Seth, P. (1999) Cytochrome P450 (P450) isoenzyme specific dealkylation of alkoxyresorufins in rat brain microsomes. *Molecular and Cellular Biochemistry,* 200, 169-76.

Dutton, D.R. & Parkinson, A.R. (1989) Reduction of 7-alkoxyresorufins by NADPH-cytochrome P450 reductase and its differential effects on their O-dealkylation by rat liver microsomal cytochrome P450. *Archives of Biochemistry and Biophysics,* 268, 617-629.

Eugster H-P., Probst M., Wurgler F.E., & Sengstang . (1993) Caffeine, estradiol, and progesterone interact with human CYP1A1 and CYP1A2. Evidence from cDNA-directed expression in *Saccharomyces cerevisiae. Drug Metabolism and Disposition,* 21, 43-49.

Farin, F., & Omiecinski C., (1993) Regiospecific expression of cytochrome P450s and microsomal epoxide hydroxylase in human brain tissue. *Journal of Toxicology and Environmental Health,* 40, 328-341.

Farin, F., Pohlman, T.,& Omiecinski, C., (1994) Expression of cytochrome P450s and microsomal epoxide hydrolase in primary cultures of human umbilical vein endothelial cells. *Toxicology and Applied Pharmacology,* 292, 1008-1014.

Hakkola, J., Pasanen M., & Raunio, H. (1996a) Expression of xenobiotic-metabolizing cytochrome P450 forms in human full-term placenta. *Biochemical Pharmacology,* 51, 403-411.

Hakkola, J., Raunio, H., & Pasanen, M. (1996b) Detection of Cytochrome P450 gene

expression in human placenta in first trimester of pregnancy. *Biochemical Pharmacology,* 52, 379-83.

Iba,M., Fung J., & Pak Y. (1999) Dose-dependent up-regulation of rat pulmonary, renal, and hepatic cytochrome P-450 (CYP) 1A expression by nicotine feeding. *Drug Metabolism and Disposition,* 27, 977-982.

Kenagy, J., Soares, M., & Audus, K. (1998) Gestational and smoking effects on peptidase activity in the placenta. *Peptides,* 19, 1659-66.

Khera, K.S. (1972) Pre-and postnatal studies on 2,4,5-trichlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid and their derivatives in rats. *Toxicology and Applied Pharmacology*, 22, 14-28

Lee, C., & Riddick D.S. (2000) Transcriptional suppression of cytochrome P450 2C11 gene expression by 3-methylcholanthrene. *Biochemical Pharmacology,* 59, 1417-1423.

Liu, F., Soares, M., & Audus, K. (1997) Permeability properties of monolayers of the human trophoblast cell line BeWo. *American Journal of Physiology,* 273, C1596-604.

Madan, A., Dehaan R., & Mudra, D. (1999) Effect of cryopreservation on cytochrome P-450 enzyme induction in cultured rat hepatocytes. *Drug Metabolism and Disposition,* 27, 327-335.

Mathis, J.M., Prough, R.A., Hines, R.N., Bresnick, E. & Simpson, E.R. (1986) Regulation of cytochrome P-450c by glucocorticoids and polycyclic aromatic hydrocarbons in cultured fetal rat hepatocytes. *Archives of Biochemistry and Biophysics*, 246, 439-448.

McDonnell, W., Scheiman, J., & Traber, P., (1992) Induction of cytochrome P4501A genes (CYP1A)by omperazole in the human alimentary tract. *Gastroenterology,* 103, 1509-1516.

Nakajima, M., Ohyama, K., & Yokoi, T. (1999) Inhibitory effects of azelastine and its metabolites on drug oxidation catalyzed by human cytochrome P-450 enzymes. *Drug Metabolism and Disposition,* 27, 792-797.

Nemoto,N., & Sakurai, J. (1995) Glucocorticoid and sex hormones as activating or modulating factors for expression of Cyp2b-9 and Cyp2b-10 in the mouse liver and hepatocytes. *Archives of Biochemistry and Biophysics,* 319, 286-292.

Nerurkar, P.V., Park S.S., & Lubet, R.A. (1993) Methoxyresorufin and Benzyloxyresorufin: Substrates Preferentially Metabolized By Cytochromes P4501A2 and 2B, Respectively, In The Rat and Mouse. *Biochemical Pharmacology,* 46, 933-943.

Pasanen, M., Taskinen, T., Sotaniemi, E.A., Kairaluoma, M. & Pelkonen, O. (1988) Inhibitor Panel Studies of Human Hepatic and Placental Cytochrome P-450 Associated Monooxygenase Activities. *Pharmacology & Toxicology,* 62**,** 311-317.

Pasanen, M., & Pelkonen, O. (1990) Xenobiotic and steroid-metabolizing monooxygenases catalysed by cytochrome P450 and glutathione s-transferase conjugations in the human placenta and their relationships to maternal cigarette smoking. *Placenta,* 11, 75-82.

Pasanen, M. & Pelkonen, O. (1994) The expression and environmental regulation of P450 enzymes in Human Placenta. Critical Reviews in Toxicology, 24, 211-229.

Pasanen, M., Haaparanta T., & Sundin M. (1990) Immunochemical and molecular biological studies on human placenta cigarette smoke-inducible cytochrome P450-dependent monooxygenase activities. *Toxicology,* 62, 175-187.

Pattillo, R.A. and Gey, G.O. (1968) The establishment of a cell line of human hormone-synthesizing trophoblastic cell in vitro. *Cancer Research,* 28,1231-1236.

Pattillo, R.A., Hussa, R.O., Ruckert, A.C., Kurtz, J.W., Cade, J.M. & Rinke, M.L. (1979) Human chorionic gonadotropin in BeWo trophoblastic cells after 12 years in continuous culture: retention of intact human chorionic gonadotropin secretion in mechanically versus enzyme-dispersed cells. *Endocrinology,* 105, 967-974.

Pichard, L., Fabre, I., & Fabre, G. (1990) Cyclosporin A drug interactions: Screening for inducers and inhibitors of cytochrome P-450 (cyclosporin A oxidase) in primary cultures of human hepatocytes and in liver microsomes. *Drug Metabolism and Disposition,* 18, 595-606.

Prough, R.A., Linder, MW., Pinaire, J.A., Xiao, G.H., & Falkner, K.C. (1996) Hormonal regulation of hepatic enzymes involved in foreign compound metabolism. *FASEB*, 10, 1369-1377.

Sakuma, T ., Ohtake, M., & Nemoto, N. (1999) Induction Of CYP1A2 By Phenobarbital In The Livers Of Aryl Hydrocarbon- Responsive And –Nonresponsive Mice. *Drug Metabolism and Disposition,* 27, 379-384.

Sesardic, A. R., Boobis, B.P. & Davies, D. (1990a) Furafylline is a potent and selective inhibitor of cytochrome P4501A2 in man. *British Journal of Clinical Pharmacology*, 29, 651-663.

Sesardic, D., Pasanen, M., & Pelkonen, O., (1990b) Differential expresssion and regulation of the cytochrome P450IA gene subfamily in human tissues. *Carcinogensis,* 11, 1183-1188.

Shimada, T., Gillam E., & Sutter T.R. (1997) Oxidation of xenobiotics by recombinant human cytochrome P450 1B1. *Drug Metabolism and Disposition,* 29, 617-622.

Shum, S., Jensen, N.M., & Nebert, D.W. (1979) The murine Ah locus: in utero toxicity and teratogenesis associated with genetic differences in benzo[a]pyrene metabolism. *Teratology*, 20, 365-76.

Sindhu, R., Mitsuhashi, M., & Kikkawa, Y. (2000) Induction of cytochrome P-450 1A2 by tryptophan in Hepa IcIc7 cells. *The Journal of Pharmacology and Experimental Therapeutics,* 292, 1008-1014.

Vakharia, D., Liu, N., & Kaminsky, L., (2001) Polycyclic aromatic hydrocarbon/metal mixtures: effect on PAH induction of CYP1A1 in human HEPG2 cells. *Drug Metabolism and Disposition,* 29, 999-1006.

Zevin, S., & Benowitz, N. (1999) Drug interactions with tobacco. An update. *Clinical Pharmacokinetics,* 36, 425-38.

Avery, M.L., Meek, C.E., and Audus, K.L. (2003) The presence of inducible cytochrome P450 types 1A1 and 1A2 in the BeWo cell line. Placenta 24, 45-52. PMID: 12495659. Publisher's official version: <http://dx.doi.org/10.1053/plac.2002.0876>. Open Access version: http://kuscholarworks.ku.edu/dspace/.

FIGURE LEGENDS

Figure 1. Induction of CYP1A1 in microsomes prepared from either BeWo cells or primary cultures of human cytotrophoblasts isolated from placentas of non-smokers. 7-Ethoxyresorufin *O*-dealkylase activity was measured after 24 hours pretreatment with indicated concentrations of either 3-methylchloranthrene (3-MC) or β-naphthoflavone (β-NF). Data points represent the mean ± the standard deviation for at least three different preparations of microsomes. Solid diamonds: BeWo cells induced with 3-MC; solid squares: primary cultures of cytotrophoblasts induced with 3-MC; solid triangles: primary cultures of cytotrophoblasts induced with β–NF.

Figure 2. Induction of CYP1A1 in BeWo cell microsomes after 24 hour pretreatment of the cells with dexamethasone, β-naphthoflavone, or the combination of the agents. Data points represent the mean ± the standard deviation for 7-Ethoxyresorufin *O*-dealkylase activity in at least three different preparations of microsomes.

Figure 3. **(A)** Immunoblot of CYP1A1/2 microsomal protein (5.0 μg) isolated from whole human placenta of nonsmokers (Lane 1), smokers (Lane 2), 8-day old primary cultures of cytotrophoblasts isolated from nonsmokers (Lane 3), and 0.5 μg rat liver (lane 4) . **(B)** Immunoblot of CYP1A1/2 microsomal protein (5.0 μg) isolated from primary cultures of cytotrophoblasts from human placenta of nonsmokers induced with 10 μM 3-methylcholanthrene for 24 hours (Lane 1), primary cultures of cytotrophoblasts from smokers (Lane 2), 0.5 μg rat liver induced with 3-methylcholanthrene (Lane 3), and human liver (Lane 4).

Figure 4. Immunoblot of microsomal protein (5.0 μg) isolated from BeWo cells exposed 24 hours to selected inducers. Lane 1, rat liver induced with 3-methylchloranthrene; Lane 2, BeWo cells treated with inducer solvent, DMSO; Lane 3, BeWo cells induced with 3-methylchloranthrene; Lane 4, BeWo cells induced with 1,2-benzanthracene; and Lane 5, BeWo cells induced with α-naphthoflavone.

Figure 5. Induction of CYP1A1 in BeWo cell microsomes after 24 hour pretreatment of the cells with 1,2-benzanthracene. Data points represent the mean ± the standard deviation for 7-Ethoxyresorufin *O*-dealkylase activity in at least four different preparations of microsomes.

Figure 6. Concentration-dependent inhibition of 1,2-benzanthracene induced CYP1A1 and CYP1A2 in BeWo cell microsomes by either α-naphthoflavone or furafylline. Enzymes, 7-ethoxyresorufin *O*-

dealkylase (EROD) and 7-methoxyresorufin *O*-dealkylase (MROD) were induced by a 24 hour pretreatment of the cells with 1,2-benzanthracene. Data points represent the mean ± the standard deviation of control EROD and MROD activity remaining following inhibition by either α-naphthoflavone and furafylline, respectively, in at least four different preparations of microsomes. The IC_{50}'s for α-naphthoflavone and furafylline were approximately 2.4 µM and 12.8 µM, respectively.

Avery, M.L., Meek, C.E., and Audus, K.L. (2003) The presence of inducible cytochrome P450 types 1A1 and 1A2 in the BeWo cell line. Placenta 24, 45-52. PMID: 12495659. Publisher's official version: <http://dx.doi.org/10.1053/plac.2002.0876>. Open Access version: http://kuscholarworks.ku.edu/dspace/.

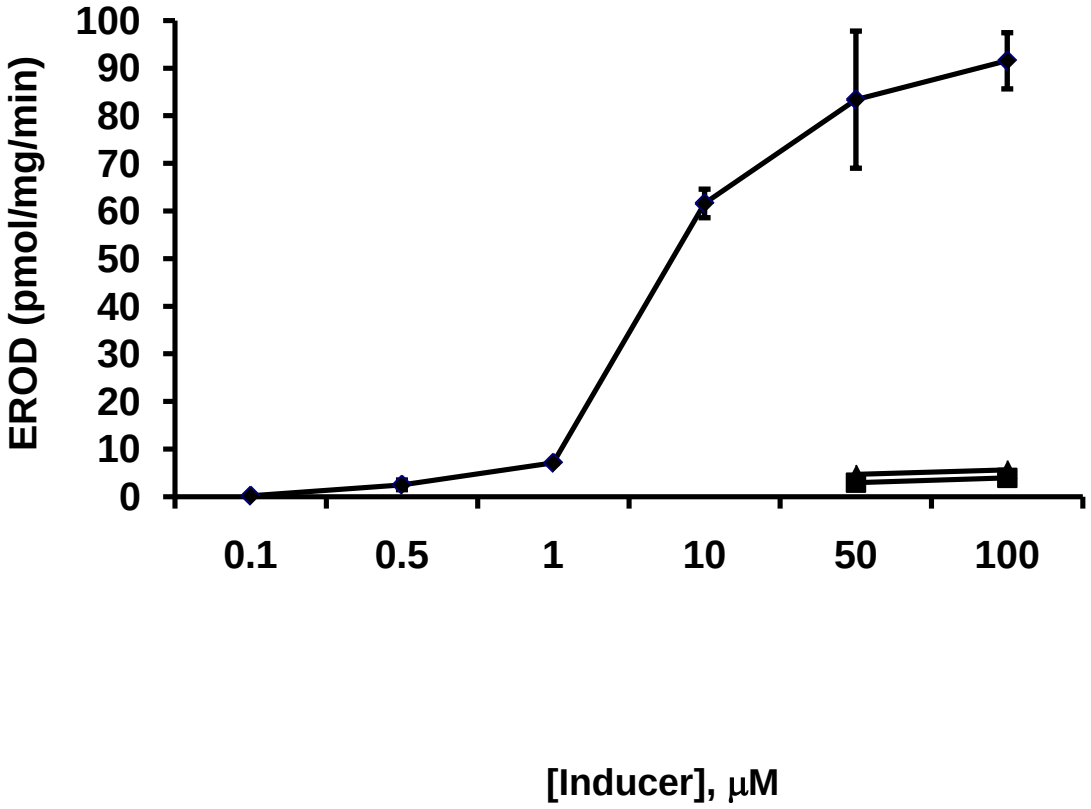
Table 1. Activity of 7-ethoxyresorufin deethylases (EROD) and 7-methoxyresorufin *O*-dealkylase (MROD) in microsomes prepared from human liver, placenta, and human cell culture systems.

		Resorufin (pmol/mg/min)	
Microsomes	**Samples**	**EROD**	**MROD**
Human liver	n = 11	42.5 ± 0.6*	39.6 ± 2.5*
Human placenta from smokers	n = 4	46.0 ± 4.4*	5.4 ± 0.5*
Human placenta from smokers	n = 4	18.9 ± 0.6*	14.7 ± 1.1*
Human placenta from nonsmokers	n = 3	3.4 ± 1.7	3.7 ± 1.4
Human placenta from nonsmokers	n = 3	1.7 ± 0.7	2.4 ± 0.6
Primary cultures of human cytotrophoblasts	n = 3	2.48 ± 0.4	-
BeWo cell monolayers	n = 3	0.45 ± 0.01*	0.40 ± 0.02*

Experiments were carried out with samples taken from indicated numbers (n) of different tissues or monolayers. The data represent the means ± standard deviation. * $P < 0.05$, data significantly different form both pools of data for human placenta from nonsmokers.

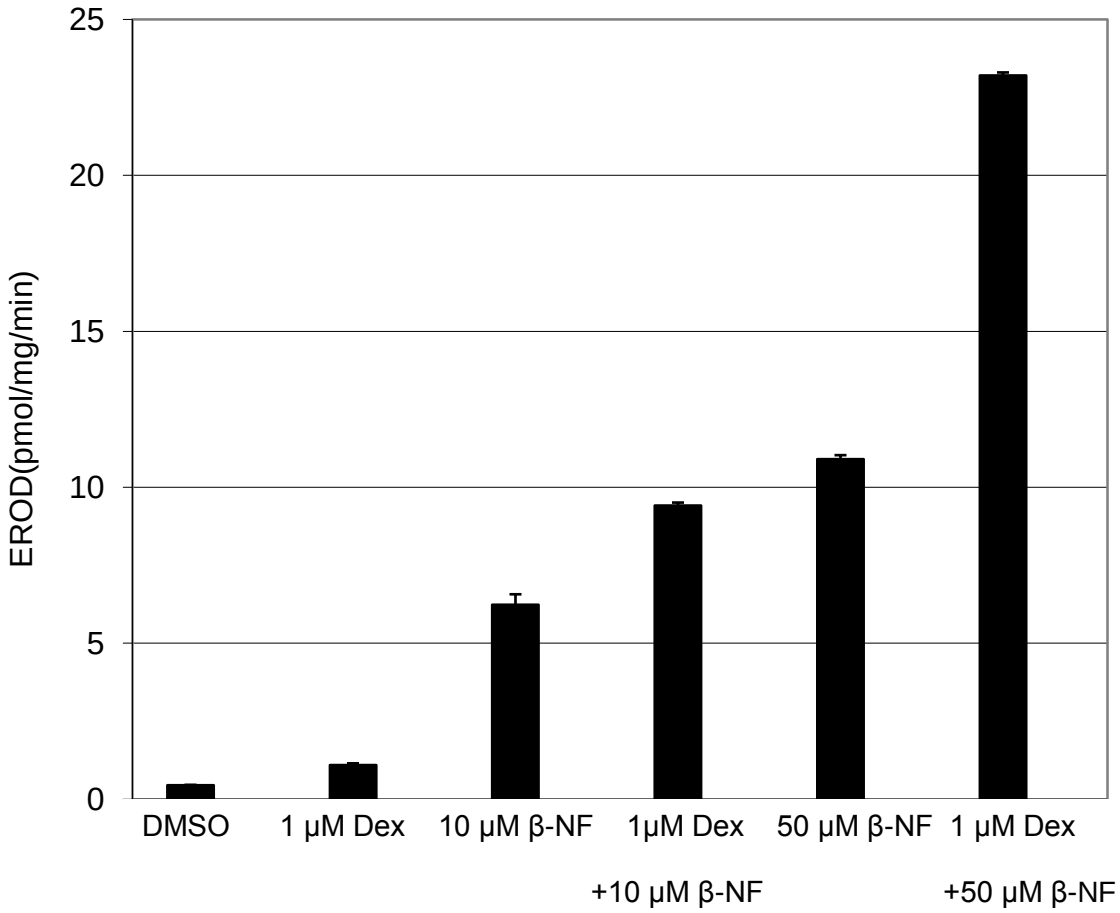
Avery, M.L., Meek, C.E., and Audus, K.L. (2003) The presence of inducible cytochrome P450 types 1A1 and 1A2 in the BeWo cell line. Placenta 24, 45-52. PMID: 12495659. Publisher's official version: <http://dx.doi.org/10.1053/plac.2002.0876>. Open Access version: http://kuscholarworks.ku.edu/dspace/.

Figure 1, Avery et al.



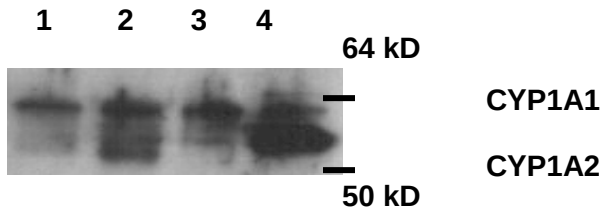
Avery, M.L., Meek, C.E., and Audus, K.L. (2003) The presence of inducible cytochrome P450 types 1A1 and 1A2 in the BeWo cell line. Placenta 24, 45-52. PMID: 12495659. Publisher's official version: <http://dx.doi.org/10.1053/plac.2002.0876>. Open Access version: http://kuscholarworks.ku.edu/dspace/.

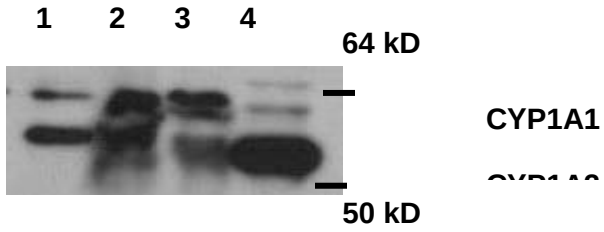
Figure 2, Avery et al.



Avery, M.L., Meek, C.E., and Audus, K.L. (2003) The presence of inducible cytochrome P450 types 1A1 and 1A2 in the BeWo cell line. Placenta 24, 45-52. PMID: 12495659. Publisher's official version: <http://dx.doi.org/10.1053/plac.2002.0876>. Open Access version: http://kuscholarworks.ku.edu/dspace/.

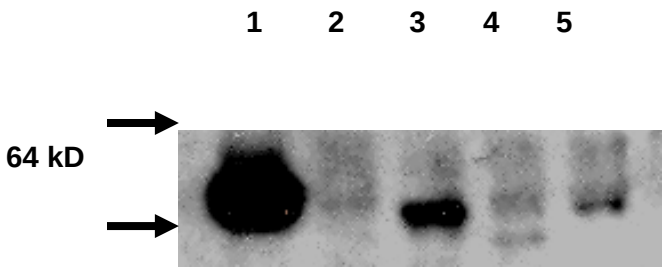
Figure 3, Avery et al.





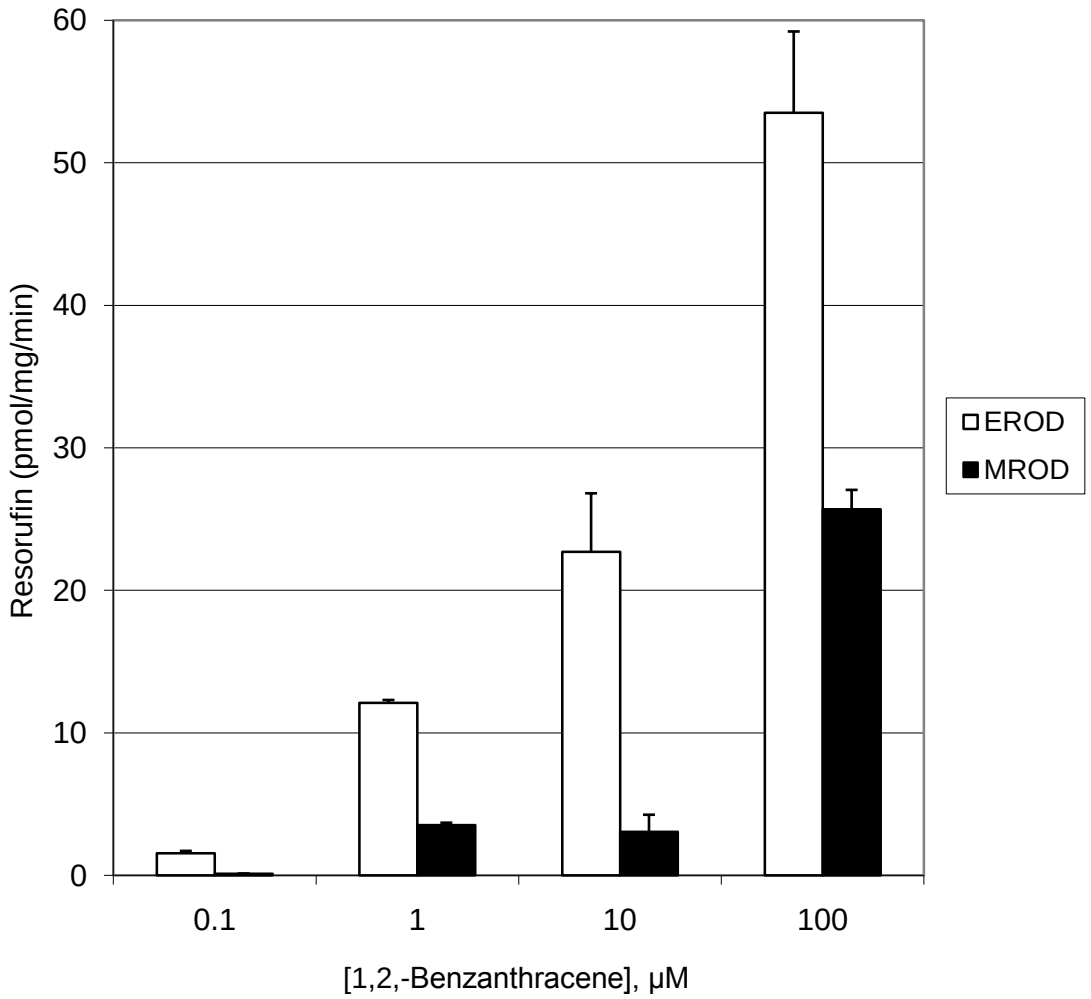
Avery, M.L., Meek, C.E., and Audus, K.L. (2003) The presence of inducible cytochrome P450 types 1A1 and 1A2 in the BeWo cell line. Placenta 24, 45-52. PMID: 12495659. Publisher's official version: <http://dx.doi.org/10.1053/plac.2002.0876>. Open Access version: http://kuscholarworks.ku.edu/dspace/.

Figure 4, Avery et al.



Avery, M.L., Meek, C.E., and Audus, K.L. (2003) The presence of inducible cytochrome P450 types 1A1 and 1A2 in the BeWo cell line. Placenta 24, 45-52. PMID: 12495659. Publisher's official version: <http://dx.doi.org/10.1053/plac.2002.0876>. Open Access version: http://kuscholarworks.ku.edu/dspace/.

Figure 5, Avery et al.



Avery, M.L., Meek, C.E., and Audus, K.L. (2003) The presence of inducible cytochrome P450 types 1A1 and 1A2 in the BeWo cell line. Placenta 24, 45-52. PMID: 12495659. Publisher's official version: <http://dx.doi.org/10.1053/plac.2002.0876>. Open Access version: http://kuscholarworks.ku.edu/dspace/.

Figure 6, Avery et al.