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TITLE PAGE

COMPARISON OF IMMORTALIZED Fa2N-4 CELLS AND HUMAN HEPATOCYTES AS *IN VITRO* MODELS FOR CYTOCHROME P450 INDUCTION

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Non standard abbreviations:

PXR, pregnane-X receptor; CYP, cytochrome P450; MDR, multidrug resistance; Pgp, P-glycoprotein; UGT, uridine 5'-diphosphate glucuronosyltransferases; NR, nuclear receptors; SHP, small heterodimer partner; NCoR, nuclear receptor corepressor; SRC, steroid receptor coactivator; NRIP, nuclear receptor interacting protein; PGC-1, peroxisome-proliferator-activated receptor-gamma coactivator; FKHR, Forkhead transcription factor FKHR (FOXO1); HNF, hepatocyte nuclear factor; Q-PCR, quantitative real-time PCR; CAR, constitutive androstane receptor; OATP; Organic Anion Transporting Polypeptide.

ABSTRACT

Fa2N-4 cells have been proposed as a tool to identify CYP3A4 inducers. To evaluate whether Fa2N-4 cells are a reliable surrogate for cryopreserved human hepatocytes, we assessed the basal mRNA expression of 64 drug disposition genes in Fa2N-4 cells. Significant differences were found in the expression of major drug metabolizing enzymes, nuclear receptors and transporters between both cell types. Importantly, the expression of constitutive androstane receptor (CAR) and several hepatic uptake transporters were significantly lower (>50-fold) in Fa2N-4 cells, while the expression of pregnane-X-receptor (PXR) and aryl hydrocarbon receptor (A*h*R) were similar between Fa2N-4 cells and human hepatocytes. Using an optimized induction assay for Fa2N-4 cells, CYP3A4 induction by rifampicin, the prototypical PXR-activator, increased from 1.5-to 7-fold at the level of functional activity. With nine selected compounds, which are known inducers of CYP3A4 either via activation of PXR, CAR, or both, we evaluated CYP3A4 and CYP2B6 mRNA induction using Fa2N-4 cells and human hepatocytes. No response was observed in Fa2N-4 cells treated with the selective CAR activators CITCO and artemisinin. CYP3A4 and -2B6 induction in Fa2N-4 cells were also low for phenytoin, phenobarbital, and efavirenz, which are dual activators of PXR/CAR. This was in agreement with the lack of expression of CAR. The EC_{50} value for rifampicin-mediated CYP3A4 induction was 10-fold higher than in human hepatocytes. This could be attributed to the low expression of hepatic organic anion transporting polypeptide OATP1B1 and OATP1B3 in Fa2N-4 cells. In summary, our findings identify limitations of Fa2N4-cells as a predictive induction model.

INTRODUCTION

Drug metabolism occurs primarily in the liver via Cytochrome (CYP) P450 enzymes. CYP3A4 is one of the predominant CYP isoforms expressed in adult liver. It catalyzes the metabolism of more than 50% of clinically used drugs (Schuetz, 2004). Induction of CYP3A4 can result in clinically significant drug-drug interactions (DDIs) or autoinduction (Lin, 2006) and therefore, is a major concern for the pharmaceutical industry.

Significant progress has been made in understanding the regulation of drug metabolizing enzymes. Two members of the gene superfamily of nuclear hormone receptors (Mangelsdorf et al., 1995; Schuetz et al., 1993), namely the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR), were identified as key transcription factors in hepatic CYP induction by xenobiotics (Kliewer et al., 1999; Blumberg et al., 1998). These nuclear receptors function as ligand-dependent transcription factors by binding to specific DNA sequences called response elements within the promoter region of genes such as CYP3A4. PXR and CAR heterodimerize with the retinoid X receptor (RXR) and bind to the above response elements and activate target gene expression (Mangelsdorf et al., 1990). Recent studies demonstrated a considerable overlap of these two receptors both in terms of the spectrum of genes regulated and their affinity to DNA-response elements (Chen et al., 2005).

A number of *in vitro* models have been developed to assess the induction potential of drug candidates. Nuclear receptor based assays, such as PXR assays, have the potential of screening large numbers of compounds which are activators of PXR (Ogg et al., 1997). However, they run the risk of false negatives because multiple mechanisms,

e.g., non-PXR mediated activation, may contribute to the induction of drugs or induction caused by metabolites not formed in cell lines. Primary or cryopreserved human hepatocytes have been considered as the standard *in vitro* models to evaluate the propensity of drug candidates to cause induction (Hewitt et al., 2007). The utility of such hepatocytes is however restricted by the limited and erratic supply, and the significant interindividual variability in the expression of drug metabolizing enzymes (Hewitt et al., 2007). These limitations have led to the search for alternative systems.

Recently, Fa2N-4, an SV40 immortalized human hepatocyte cell-line, has been developed by Multicell Technologies (Mills et al., 2004). Initial studies by Mills et al. (2004) have shown that multiple CYP enzymes, including CYP3A4, CYP1A2, and CYP2C9, are inducible in Fa2N-4 cells after exposure to several prototypical inducers. In addition, the expression of PXR and A*h*R, which are important transcription factors involved in the regulation of drug metabolizing enzymes and transporters, have been detected in Fa2N-4 cells (Mills et al., 2004). Ripp et al. (2006) further characterized Fa2N-4 cells by evaluating the effect of selected compounds on CYP3A4 induction and comparing *in vitro* induction data with *in vivo* induction response. It was suggested that Fa2N-4 cells could be used to identify CYP3A4 inducers as well as to predict the magnitude of clinical DDIs. Given that Fa2N-4 cells have a similar morphology to human hepatocytes, maintain the inducibility of various drug metabolizing enzymes, and are easily maintained and propagated, Fa2N-4 cells have been proposed as a tool to identify CYP3A4 inducers in drug discovery and development. In the Draft "Guidance for Industry" issued by The Food and Drug Administration (FDA) (http://www.fda.gov/cder/guidance/6695dft.pdf), the use of immortalized cells, as a CYP

induction model, is considered acceptable provided that it can be demonstrated with positive controls that CYP3A4 is inducible in these cell-lines.

However, it remains unclear if Fa2N-4 cells are a reliable surrogate for primary or cryopreserved human hepatocytes. For instance, CYP3A4 induction by selective CAR activators was not evaluated in Fa2N-4 cells (Ripp et al., 2006). Furthermore, the expression of major drug metabolizing enzymes, nuclear receptors, and hepatic transporters in these cells are also not known. This information is critical to understand the molecular mechanism of CYP3A4 induction in Fa2N-4 cells.

The objectives of our studies were: 1) to compare the basal expression of drug disposition genes in Fa2N-4 cells and cryopreserved human hepatocytes, 2) to optimize the culture and induction conditions to attain a good CYP3A4 functional activity window, and 3) to compare CYP3A4 and CYP2B6 induction potency in Fa2N-4 cells and human hepatocytes for selected compounds which are known inducers of CYP3A4 either via potential activation of PXR or CAR, or both.

MATERIALS AND METHODS

Materials

Rifampicin, artemisinin, phenytoin, phenobarbital and CITCO were purchased from Sigma-Aldrich (St. Louis, MO). All other compounds were synthesized by the Merck Chemistry Department (West Point, PA) and were of the highest purity. RNeasy 96 Kits were purchased from Qiagen (Valencia, CA). The Quant-iT Ribogreen RNA Reagent Kit was purchased from Molecular Probes, Invitrogen Detection Technologies (Carlsbad, CA). Universal PCR Master Mix and Low Density Microarrays were purchased from Applied Biosystems (Foster City, CA). Collagen-coated 48-well plates were purchased from BD Biosciences (San Jose, CA). All other reagents and chemicals were of the highest grade and purchased from either Fisher Scientific (Pittsburg, PA) or Sigma-Aldrich (St. Louis, MO).

Cells

Cryopreserved Fa2N-4 cells, MFE^{TM} support medium and MFE^{TM} plating medium were purchased from XenoTech LLC. (Lenexa, KS). Cryopreserved human hepatocytes (LHO - female Caucasian 68-years-old; 455 - female Caucasian 37-years-old; 527 – female Caucasian 36-years-old; and DMQ - African-American female 59-years-old), antibiotic/antimycotic Torpedo Mix, In VitroGRO-CP Media and In VitroGRO-HI incubation Media were obtained from *In Vitro* Technologies (Baltimore, MD) or XenoTech LLC (Lenexa, Kansas), respectively.

Optimization of CYP3A4 and CYP1A2 induction in Fa2N-4 Cells

CYP3A4 and CYP1A2 induction studies in Fa2N-4 cells were optimized from the manufacturers' protocol (http://www.multicelltech.com/pdfs/5.pdf). CYP3A4 and CYP1A2 induction was assessed based upon both mRNA expression and enzymatic activity. Rifampicin and omeprazole, the prototypical PXR and A*h*R activators (LeCluyse, 2001; Yueh et al., 2005), were used to evaluate CYP3A4 or CYP1A2 induction. Testosterone (250 µM) or phenacetin (100 µM) was selected as the probe substrate to measure CYP3A4-mediated testosterone 6ß-hydroxylation and CYP1A2-mediated phenacetin de-ethylation, respectively. In our studies, the following conditions were evaluated at both mRNA and activity level in order to increase the induction window: A) Cells were plated at different plating densities ranging from 75 000 to 130 000 cells per well, B) The probe substrate incubation period was assessed over a period ranging from 1-to-4 hours, C) CYP induction was assessed in the presence and absence of the 48 hour adaptation period, and D) Functional activity was compared between 48 and 72 hour drug treatment. The optimized protocol is described in detail below.

Cell culture and induction studies of Fa2N-4 Cells and cryopreserved human hepatocytes

Fa2N-4 cells (95 000 cells/well) or human hepatocytes (120 000 cells/well) were plated in collagen coated 48-well plates. Cells were maintained at 37^{o}C, 95% humidity, and 5% CO_2. After cell attachment (approximately 3 hours), serum-containing plating medium was replaced with serum-free culture medium. Fa2N-4 cells were incubated for 48 hours (adaptation period) before treating with test compounds. This adaptation was

necessary for the cells to exhibit a robust induction response. Cryopreserved human hepatocytes were allowed an adaptation period of 24 hours. Stock solutions of test compounds were prepared in dimethyl sulfoxide (DMSO) and diluted 1000-fold with serum-free medium for drug treatment. After 24 hours of treatment with test compounds, or vehicle control (0.1% DMSO, v/v), medium was discarded and replaced with fresh medium containing test compounds. This was repeated every 24 hours during the 48 hour drug incubation period for human hepatocytes, and the 72 hour drug incubation period for Fa2N-4 cells. At the end of the incubation, drug-containing medium was discarded and the cells were washed with drug-free medium. Cells were then exposed to the probe substrates testosterone 250 µM (CYP3A4) or phenacetin 100 µM (CYP1A2) for 1 hour. The media was collected and the metabolites produced by testosterone 6ß-hydroxylation or phenacetin de-ethylation were quantitated using LC/MS-MS as described previously (Prueksaritanont et al., 2005). The remaining cells were used to isolate mRNA, which was subjected to quantitative-PCR to assess changes in CY3A4, CYP2B6 and CYP1A2 mRNA expression. Rifampicin (10 µM) was used as the positive control for both CYP3A4 and CYP2B6, while omeprazole (50 µM) functioned as the positive control for CYP1A2.

Cytotoxicity Assessment

Cytotoxicity studies were conducted in parallel with the WST-1 Cell Proliferation kit (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's protocol. Briefly, after the 72 hr drug treatment period, WST1 reagent was added to cell culture medium (1 part reagent to 10 parts culture medium), and mixed gently on an orbital

shaker for 1 minute. Cells were then returned to the incubator for 2 hours (37^0C, 5% CO_2). Thereafter, plates were shaken gently on an orbital shaker for 1 min, and absorbance was measured at 450 nm. Wells containing culture medium, but no cells, were used to determine background values. Treatments were deemed toxic if there was a significant decrease in absorbance compared with vehicle treatment.

Reverse-Transcription Quantitative PCR

Total RNA was isolated using the RNeasy 96 Kit and quantitated using the Quant-iT Ribogreen RNA Reagent Kit. A two-step reverse transcriptase (RT)-PCR reaction was conducted by reverse transcribing 50 ng of total RNA to cDNA using TaqMan® Reverse Transcription Reagents, according to the TaqMan® Universal PCR Master Mix protocol. PCR reactions were then prepared by adding an aliquot of cDNA (3 µl) to a reaction mixture containing the TaqMan® Fast Universal PCR Master Mix solution, primers, and probes for CYP3A4/CY2B6/CYP1A2. Low density microarray studies to assess the basal expression of drug-disposition genes were conducted by adding cDNA (50 µl) to TaqMan® Universal PCR Master Mix solution. Low-density microarrays (microfluidic cards) were custom-made and validated by Applied Biosystems and contained probes in triplicate for the detection of 64 genes (see Tables 1, 2 and 3). PCR amplified cDNAs were detected by real-time fluorescence on an ABI PRISM 7900 Fast Sequence Detection System (Perkin Elmer, Wellesley, MA).

Data Analysis

Quantitation of the target cDNAs, to assess basal gene expression, were normalized to 18S ribosomal RNA (Ct_{target} - Ct_{18S} = ΔCt), and the difference in expression for each target cDNA in the human hepatocytes was expressed relative to the amount in Fa2N-4 cells ($\Delta Ct_{human\ hepatocytes} - \Delta Ct_{Fa2N} = \Delta\Delta Ct$). Fold changes in target gene expression were determined by taking 2 to the power of this number ($2^{-\Delta\Delta Ct}$). Statistical analysis of the differences in basal gene expression between Fa2N-4 cells and human hepatocytes were based upon differences in the ΔCt values (Pfaffl, 2001).

Student's *t* tests were used to determine the significance of differences between measurements and differences with *P* values <0.05 were considered significant.

To determine the EC_{50} and E_{max} values, the data from concentration response curves were fitted to a three-parameter sigmoid (Hill) model, according to the following equation:

$$y = (E_{max} \cdot x^{\gamma})/(EC_{50}^{\gamma} + x^{\gamma}) \qquad \text{Equation (1)}$$

All curve-fitting was carried out using SigmaPlot 10.0 (Systat Software, Inc., Chicago, IL). The studies conducted with Fa2N-4 cells represent the mean of three independent experiments done in triplicate. Studies conducted in human hepatocytes (LHO, 455 and 527) represent the mean of triplicate wells, while studies conducted in DMQ represent the mean of duplicate wells.

RESULTS

Changes in the expression of drug disposition genes in cultured Fa2N-4 Cells and cryopreserved human hepatocytes after an adaptation period

Given the fact that some drug disposition genes, including uptake and efflux transporters, as well as transcription factors could influence the induction of CYP3A4 (Lam et al., 2006), we evaluated the basal expression of 64 drug disposition genes (listed in Tables 1-3) in Fa2N-4 cells and compared those to four batches of cryopreserved human hepatocytes. Genes analyzed included Phase I and II drug metabolizing enzymes, hepatic transporters, transcription factors, and the coactivators and corepressors involved in the regulation of the aforementioned genes.

We first assessed whether gene expression levels changed during the 48 and 24 hour adaptation periods for Fa2N-4 cells and cryopreserved human hepatocytes, respectively. In Fa2N-4 cells, the expression levels of all 64 genes increased after the 48 hour adaptation period. We observed a >10-fold increase for 19 genes (Tables 1-3, denoted by [a]). The largest increases in expression were observed for CYP2B6 (~67-fold) and GSTA2 (~82-fold). In contrast, in most cases, gene expression levels in human hepatocytes decreased (2-4-fold) or remained the same after the 24 hr adaptation period (data not shown). In human hepatocytes, the genes which showed larger than 10-fold increases were limited to ABCC2, CYP3A5 and UGT1A6 for LHO; SLCO1B1, CYP2C19 and NR1H4 for DMQ; and ABCG2 for 455 (Tables 1-3, denoted by [b]).

Basal expression of drug disposition genes in Fa2N-4 cells compared to human hepatocytes

After determining the impact of the adaptation period on gene expression in Fa2N-4 cells and human hepatocytes, we assessed the differences in gene expression prior to the initiation of drug treatment, which was defined as basal expression, between the human hepatocytes and Fa2N-4 cells. Significant differences were observed for all categories of genes including phase I and II enzymes, transporters, as well as transcription factors and coactivators/corepressors (Tables 1-3).

As shown in Table 1, in four batches of cryopreserved hepatocytes tested, the CYP3A4 expression levels, compared to Fa2N-4 cells, ranged from 0.3- to 18-fold. CYP1A2 expression levels were significantly higher in all four batches of hepatocytes (19.3 - >100-fold) when compared to Fa2N-4 cells. Similarly, CYP2D6, CYP2E1, CYP1A1, UGT1A1, UGT1A6, UGT2B15 and UGT2B4 expression levels were significantly higher in human hepatocytes compared to those in Fa2N-4 cells.

Except for NROB2 (small heterodimer partner, SHP), NR1I3 (CAR) and VDR (vitamin D receptor), Fa2N-4 cells appear to have higher expression of most transcription factors and coactivators/corepressors which have been associated with PXR or CAR-mediated enzyme induction (Moore et al., 2006; Table 2). The expression of NR1I2 (PXR) was slightly higher in Fa2N-4 cells compared to three batches of hepatocytes (455, 527 and LHO), but lower than in DMQ (~2.5-fold) (Table 2). Interestingly, the expression of NR1I3 (CAR) in Fa2N-4 cells (Ct value ~ 35) was nearly two orders of magnitude lower than expression in all four batches of human hepatocytes (Table 2). The expression of the A*h*R and NR3C1 (glucocorticoid receptor), which are involved in

the regulation of CYP1A2 and CYP3A4 (Tirona and Kim, 2005), respectively, were significantly lower in all four batches of human hepatocytes compared to Fa2N-4 cells. NROB2 (small heterodimer partner, SHP) showed much higher expression in all four batches of hepatocytes compared to Fa2N-4 cells. Conversely, the expression of TCF1 (hepatocyte nuclear factor 1, HNF1), NCOR1 (nuclear receptor corepressor 1), NCOR2 (nuclear receptor corepressor 2) and NR1H2 (liver-X receptor, LXR) were lower in human hepatocytes compared to Fa2N-4 cells (Table 2).

Expression levels of hepatic transporters were also compared between Fa2N-4 cells and cryopreserved hepatocytes (Table 3). Fa2N-4 cells demonstrated a significantly lower basal expression of several major hepatic uptake transporters, including SLC10A1 (sodium/bile acid cotransporter 10A1, NTCP), SLC22A1 (solute carrier organic cation transporter 22A1, OCT1), SLCO1B1 (solute carrier organic anion transporter 1B1, OATP1B1) and SLCO1B3 (solute carrier organic anion transporter 1B3, OATP1B3). The most significant differences were observed for SLC10A1, SLCO1B1, and SLCO1B3 (Ct value in Fa2N-4 cells was > 35), in which the expression of these genes were 50-fold higher in all four batches of hepatocytes compared to Fa2N-4 cells (Table 3). Except for SLCO1A2, the basal expression levels of all uptake transporters were highest in DMQ compared to the other three batches of human hepatocytes tested. The basal expression of several hepatic efflux transporters was also evaluated. The expression of ABCB1 (the multidrug resistance gene 1, MDR1-P-glycoprotein) was significantly lower in all four batches of human hepatocytes compared to Fa2N-4 cells, while the expression of ABCC2 (the multidrug resistance protein 2, MRP2) was ~2-5-fold ($p<0.05$) higher. The expression of ABCB11 (bile-salt exporter protein, BSEP) was approximately two orders

of magnitude greater in all four batches of hepatocytes compared to Fa2N-4 cells. The expression of ABCG2 (breast cancer resistance protein, BCRP) appears to be similar in Fa2N-4 cells and human hepatocytes. Variable gene expression was observed for all of the other uptake and efflux transporters amongst the four batches of human hepatocytes compared to Fa2N-4 cells (Table 3).

Induction of CYP3A4 and CYP1A2 in Fa2N-4 cells under optimized culture conditions

Using the manufacturer (XenoTech LLC) recommended protocol, CYP3A4 functional activity was less than 2-fold in Fa2N-4 cells treated with rifampicin 1-50 µM (Figure 1C), although we observed a concentration dependent increase in CYP3A4 mRNA expression (rifampicin 1-20 µM), which ranged from 10-to 60-fold (Figure 1A). We therefore optimized the culture and induction conditions to increase the CYP3A4 induction window at the level of functional activity as described in the Materials and Methods section.

With the optimized protocol, a dose-dependent increase in CYP3A4 functional activity was observed which ranged from 1-to 10-fold after the cells were treated with rifampicin 1-20 µM (Figure 1C), while CYP3A4 induction at the mRNA level was comparable to the data obtained using the manufacturer recommended protocol (Figure 1A). A dose-dependent increase in CYP1A2 mRNA and activity were observed in both methods when Fa2N-4 cells were treated with omeprazole (1-20 µM) (Figure 1B and 1D). At omeprazole 50 µM (the FDA recommended CYP1A2 positive control

concentration), CYP1A2 induction at both mRNA and activity levels were comparable between these two methods (Figure 1B and 1D).

Comparison of CYP3A4 induction in Fa2N-4 cells and human hepatocytes

To assess whether Fa2N-4 cells could be used as a predictive model to evaluate CY3A4 induction, nine compounds were selected to measure concentration-dependent CYP3A4 induction in Fa2N-4 cells and cryopreserved human hepatocytes (Figures 2 and 3). Among the compounds tested, rifampicin, bosentan, moricizine and ritonavir are reported as selective PXR-activators (LeCluyse, 2001; van Giersbergen et al., 2002; Luo et al., 2002), phenobarbital, phenytoin and efavirenz are PXR/CAR dual activators (Trubetskoy et al., 2005; Wang et al., 2004; Bell and Michalopoulos, 2006; Faucette et al., 2007; Hariparsad et al., 2004), while CITCO and artemisinin are selective CAR-activators (Simonsson et al., 2006; Maglich et al., 2003).

Rifampicin-mediated CYP3A4 induction was conducted in Fa2N-4 cells and four batches of human hepatocytes (DMQ, 527, 455, and LHO). Dose-response curves in Fa2N-4 cells (Figure 2A) obtained from three independent experiments were fitted to the sigmoidal model with EC_{50} values ranging between 7 to 9 μM and E_{max} ranging between 54- to 69- fold. In comparison, EC_{50} values in human hepatocytes (DMQ, 527, 455, and LHO) ranged from 0.4 μM to 1.3 μM and E_{max} values from 29- to 53-fold (Figure 2B). Rifampicin-mediated CYP3A4 induction was also evaluated at the level of CYP3A4 functional activity in Fa2N-4 cells and human hepatocytes (DMQ) (data not shown). The EC_{50} values obtained were 6.2±3.1 μM for Fa2N-4 cells and 0.3±0.2 μM for human

hepatocytes (DMQ), respectively, and the corresponding E_{max} values were 12.7±2.2 and 12.3±2.0-fold.

Dose-response studies for CYP3A4 induction were also conducted for additional compounds in Fa2N-4 cells and compared with the data obtained in one batch of human hepatocytes (DMQ) (Figure 3). Due to the poor correlation that we observed for most compounds between changes in CYP3A4 mRNA expression and functional activity, we compared the response between both cell types based upon CYP3A4 mRNA expression. As shown in Figure 3 and Table 4, the EC_{50} and E_{max} values for phenytoin and moricizine were roughly within a two-fold range between the two cell systems. Interestingly, while the EC_{50} values for bosentan were reasonably similar between the two cell systems, the E_{max} in hepatocytes was 4-fold higher in hepatocytes than in Fa2N4 cells.

[0] The EC_{50} values of two selective CAR activators, artemisinin and CITCO, were 21.4 and 0.8 μM in human hepatocytes (Table 4). However, the induction response in Fa2N-4 cells was too low to be fitted to the sigmoidal model (Figure 2D and 2E).

Dose-response curves for ritonavir could not be fitted to a sigmoidal model due to the bell shaped response (Figure 2C). A dose-dependent CYP3A4 induction was observed in both Fa2N-4 cells and human hepatocytes treated with ritonavir with a maximal response observed between 5-10 μM, after which the fold change decreased sharply. The reason for the ritonavir bell-shaped response is currently not clearly understood (Ripp et al., 2006). Data for phenobarbital also could not be fitted to the sigmoidal model because no saturable response was observed in either Fa2N-4 cells or human hepatocytes (DMQ) within the concentration range tested (Figure 2F). Human hepatocytes treated with phenobarbital at 200, 500 or 1000 μM produced 20-, 40- and 90-

fold CYP3A4 mRNA induction, while 4.7-, 10.4- and 28.3-fold induction were observed in Fa2N-4 cells treated with the same concentrations of phenobarbital. A bell-shaped response was observed in Fa2N-4 cells and human hepatocytes (DMQ) treated with efavirenz (Figure 2H). The lower induction observed at higher concentrations of efavirenz (20-50 μM) was caused by cytotoxicity. At 5 μM concentration, 1.8-fold and 22.4-fold induction was observed in Fa2N-4 cells and human hepatocytes (DMQ) treated with efavirenz.

Comparison of CYP2B6 mRNA induction in Fa2N-4 cells and human hepatocytes (DMQ)

To further confirm the absence of CAR in Fa2N-4 cells, CYP2B6 mRNA induction was compared between human hepatocytes and Fa2N-4 cells treated with selected compounds.

Rifampicin-mediated CYP2B6 induction was fitted to the sigmoidal model with an EC_{50} of 8.0 μM and an E_{max} of 4.1-fold for Fa2N-4 cells, and an EC_{50} of 2.3 μM and an E_{max} of 12.9-fold for human hepatocytes, respectively (Figure 4A, Table 4). In Fa2N-4 cells, the EC_{50} and E_{max} values for phenobarbital were 204.4 μM and 5.0-fold, respectively. Correspondingly, the EC_{50} and E_{max} values in human hepatocytes were 60.4 μM and 31.3-fold, respectively (Figure 4B, Table 4). Bosentan-mediated CYP2B6 induction was low in Fa2N-4 cells, but produced an EC_{50} value of 0.4 μM and an E_{max} of 3.8-fold in human hepatocytes (Figure 4F, Table 4). Fa2N-4 cells treated with the selective CAR-agonists artemisinin and CITCO or the dual PXR/CAR activator phenytoin produced a low response in Fa2N-4 cells and could not be fitted to the

sigmoidal model. Data from human hepatocytes (DMQ) showed a concentration-dependent response for phenytoin, artemisinin and CITCO (Figure 4C-E, Table 4). These data supported the conclusion that the CAR pathway is deficient in Fa2N-4 cells.

DISCUSSION

In this report, we performed a comprehensive assessment of the utility of Fa2N-4 cells as a CYP3A4 induction model. Our studies indicate that Fa2N-4 cells possess comparable levels of PXR and A*h*R, which are major pathways involved in the regulation of CYP3A4 and CYP1A2, respectively in human hepatocytes (Table 2 and Figure 1). However, significant differences exist in the basal mRNA expression of several drug disposition genes in Fa2N-4 cells compared to human hepatocytes. Especially, very low expression of CAR and several hepatic uptake transporters was observed in Fa2N-4 cells. This could potentially limit the application of Fa2N-4 cells as an *in vitro* tool for the (quantitative) prediction of CYP3A4 induction in the clinic.

The expression levels of PXR (NR1I2), the primary transcription factor involved in the regulation of CYP3A4 (Tirona and Kim, 2005), was similar between Fa2N-4 cells and human hepatocytes (Table 2). In addition, several coactivators and corepressors of PXR (Moore et al., 2006) such as the small heterodimer partner (SHP/NCOB2), nuclear receptor corepressor 2 (NCoR2/SMRT), steroid receptor coactivators 1 (SRC1/NCOA1), and 2 (SRC2/GRIP1), nuclear receptor interacting protein 1 (NRIP1/RIP140), peroxisome-proliferator-activated receptor-gamma coactivator (PGC-1), and Forkhead transcription factor FKHR (FOXO1) were also expressed in Fa2N-4 cells. Interestingly, the expression levels of CAR were dramatically lower ($p<0.05$) in Fa2N-4 cells compared to all four batches of cryopreserved hepatocytes (Table 2). This will have a significant impact on the induction of genes such as CYP3A4 and CYP2B6 given that there is considerable overlap of PXR and CAR both in terms of the spectrum of genes regulated and their affinity to DNA-response elements (Chen et al., 2005).

Basal expression of several hepatic uptake transporters, including OATP1B1 (SLCO1B1), OATP1B3 (SLCO1B3), NTCP (SLC10A1), and OCT1 (SLC22A1), were significantly lower in Fa2N-4 cells compared to all four batches of human hepatocytes. Uptake of drugs into the hepatocytes is achieved by both passive diffusion and carrier-mediated transport (Shitara et al., 2006; van Montfoort et al., 2003). OATP1B1 and -1B3, which are specifically expressed in the hepatocyte sinusoidal membrane, do transport several clinically used drugs, including some potent CYP3A4 inducers, such as rifampicin (Tirona and Kim, 2002). Hepatic uptake transporters, could affect the intracellular exposure of their substrates in the liver, and therefore, modulate the induction by CYP enzymes, when transporter-mediated hepatic uptake is the rate-limiting step for uptake of drugs into the liver. Given the low hepatic transporter expression in Fa2N-4 cells, the induction potential of substrates for these transporters likely will be underestimated. Basal expression of hepatic efflux transporter ABCB11 (BSEP) was also significantly lower in Fa2N-4 cells (Table 3). Relevance of the low BSEP expression to CYP3A4 induction is unclear as most BSEP substrates are not inducers of CYP3A4.

To validate whether Fa2N-4 cells could be used as a reliable model for CYP3A4 induction, we first compared the CYP3A4 mRNA induction by rifampicin, a prototypical activator of PXR, in Fa2N-4 cells and human hepatocytes (Figure 2). The E_{max} obtained from Fa2N-4 cells (~ 60-fold) was comparable to that in four batches of human hepatocytes (~ 53-fold), however, the EC_{50} in Fa2N-4 cells (~ 8 μM) was approximately 10- fold higher compared to human hepatocytes (~ 0.8 μM) (Figure 2). Similar results were also observed at the level of CYP3A4 activity (data not shown). The increased EC_{50} could be attributed to the low OATP1B1/1B3 expression in Fa2N-4 cells as

rifampicin is a substrate of these transporters (Tirona et al., 2002). Tirona et al (2002) have demonstrated that increased expression of OATP1B1 in Hela cells significantly enhanced rifampicin-mediated PXR activation. Such studies would also be needed in Fa2N-4 cells to confirm our hypothesis. Interestingly, bosentan, a PXR agonist and a substrate for OATP1B1 (Treiber et al., 2007) showed an EC_{50} value of 1.8 µM in Fa2N-4 cells which was less than 2-fold higher than in human hepatocytes DMQ (1 µM), while the E_{max} in Fa2N-4 cells (12-fold induction) was nearly 5-fold lower than in human hepatocytes (48-fold induction). The mechanism explaining this difference in E_{max} is not clear, but it might be explained by the involvement of other nuclear receptors like for instance CAR. More experiments will be needed to understand this further.

The lack of CYP3A4 and 2B6 induction for the selective CAR-agonists CITCO and artemisinin in Fa2N-4 cells confirms our observation that CAR expression is very low in Fa2N-4 cells (Figures 2 and 3, Table 2). In human hepatocytes (DMQ), the EC_{50} value for CITCO–mediated CYP2B6 induction (0.01 µM) was much lower than for CYP3A4 (0.8 µM). In addition, CITCO showed a much lower E_{max} for CYP3A4 induction compared to rifampicin. This concurs with the current literature that CYP2B6 is regulated primarily via CAR (Faucette et al., 2006).

We also evaluated CYP3A4 and 2B6 induction with several dual PXR and CAR activators, including phenytoin, efavirenz, and phenobarbital (Figures 3 and 4, Table 4). Phenytoin showed a relatively low CYP3A4 induction response in Fa2N-4 cells compared to human hepatocytes (Figure 3G). Furthermore, a dose-dependent CYP2B6 induction was observed in human hepatocytes (EC_{50} 9.8 µM), but not in Fa2N-4 cells. Studies conducted by Luo et al. (2002) and Wang et al. (2004) indicated that phenytoin is

a potent CAR, but a weak PXR activator. Therefore, the low inductive response of phenytoin in Fa2N-4 cells is likely due to the low CAR expression. Similarly, efavirenz a relatively potent CAR but weak PXR activator (Faucette et al., 2007) also showed low CYP3A4 induction in Fa2N-4 cells (Figure 3H). Although the dose-response curves for CYP3A4 mRNA induction by phenobarbital were not saturable at the maximum concentration tested (1000 μM), the CYP3A4 induction in Fa2N-4 cells at phenobarbital concentrations of 200, 500, and 1000 μM was much lower than in human hepatocytes (Figure 3F). Phenobarbital caused a low CYP2B6 induction in Fa2N-4 cells (Table 4). It was not unexpected that phenobarbital induced CYP2B6 in Fa2N-4 cells, even in the absence of CAR expression. This observation could be explained by the crosstalk between PXR and CAR in the regulation of drug disposition genes (Pascussi et al., 2008). Faucette et al (2006) observed that PXR activators induced both CYP3A4 and CYP2B6, while CAR activators induced CYP2B6 but not CYP3A4. Thus the expression of PXR in Fa2N-4 cells and human hepatocytes could cause the induction of CYP2B6 mRNA in Fa2N-4 cells treated with phenobarbital as this compound is also a PXR activator (Bell and Michalopoulos, 2006). This may also explain why rifampicin, which is a selective PXR activator, induced CYP2B6 (EC_{50} 8.0 μM, E_{max} 4.1-fold) in Fa2N-4 cells (LeCluyse, 2001). Interestingly, the EC_{50} (2.3 μM) value generated in human hepatocytes was significantly lower than that observed in Fa2N-4 cells, while the E_{max} value (12.9-fold) was significantly higher (Table 4). This further emphasizes the importance of CAR in the regulation of CYP2B6 (Faucette et al., 2006; Faucette et al., 2007).

In conclusion, we observed very low expression of CAR, a key nuclear hormone receptor for CYP3A4 and CYP2B6 induction, in Fa2N-4 cells. In addition, we also found that there is a significantly lower expression of several hepatic uptake transporters in Fa2N-4 cells. This could result in false negatives or a low level of induction for CAR activators or substrates of hepatic uptake transporters. Our studies indicate that Fa2N-4 cells cannot replace primary human hepatocytes as an *in vitro* model system to prospectively predict the induction potential of drug candidates in the clinic.

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Figure Legends

Figure 1: Induction of CYP3A4 and CYP1A2 mRNA and activity in Fa2N-4 cells. Rifampicin mediated-CYP3A4 and omeprazole mediated-CYP1A2 induction were compared using the manufacturer (XenoTech LLC) recommended protocol (white columns) and our optimized assay conditions (black columns) as described in the Materials and Methods section. A) CYP3A4 mRNA, B) CYP1A2 mRNA, C) CYP3A4 mediated testosterone 6β-hydroxylation, and D) CYP1A2 mediated phenacetin o-deethylation following treatment with various concentrations of rifampicin and omeprazole, respectively. The data were expressed as mean ± SE of the fold changes as compared to 0.1% DMSO vehicle control and obtained from three independent experiments.

Figure 2: Concentration-response curves for CYP3A4 mRNA induction in Fa2N-4 cells and human hepatocytes treated with rifampicin. Concentration-response curves obtained from A) Fa2N-4 cells [Experiment 1 (closed circles), Experiment 2 (open circles), and Experiment 3 (closed triangles) and B) human hepatocytes [527 (closed circles), 455 (open circles), LHO (closed triangles) and DMQ (open triangles) were fitted to a sigmoidal model as described in the Materials and Methods section. The data obtained from Fa2N-4 cells, 527, 455 and LHO represent the mean ± SE of one experiment performed in triplicate. The data from DMQ represent the mean of one experiment performed in duplicate.

Figure 3: Concentration-response curves for CYP3A4 mRNA induction in Fa2N-4 cells and human hepatocytes treated with several compounds. Concentration-response curves obtained from both Fa2N-4 cells (closed circles) and human hepatocytes (DMQ) (open circles) were fitted to a sigmoidal model as described in the Materials and Methods section. Data for artemisinin, CITCO in Fa2N-4 cells, efavirenz, phenobarbital or ritonavir in both Fa2N-4 cells and human hepatocytes were not fitted to the model due to the very low CYP3A4 induction, lack of a saturable response or a bell-shaped concentration response relationship. The data obtained from Fa2N-4 cells represent the mean ± SE of three independent experiments performed in triplicate. The data from human hepatocytes (DMQ) represent the mean of one experiment performed in duplicate.

Figure 4: Concentration-response curves for CYP2B6 mRNA induction in Fa2N-4 cells and human hepatocytes treated with several tested compounds. Concentration-response curves obtained from both Fa2N-4 cells (closed circles) and human hepatocytes (open circles) were fitted to a sigmoidal model as described in the Materials and Methods section. In Fa2N-4 cells, dose-response data for phenytoin, artemisinin, CITCO and bosentan could not be fitted to the sigmoidal model due to the very low observed response. The data obtained from Fa2N-4 cells represent the mean of three independent experiments performed in triplicate. The data from human hepatocytes (DMQ) represent the mean of one experiment performed in duplicate.

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Table 1: Comparison of phase I and phase II enzyme gene expression between Fa2N-4 cells and cryopreserved human hepatocytes.

The gene expression levels were evaluated in human hepatocytes and Fa2N-4 cells after the 24h and 48h adaptation periods, respectively. Data are expressed as means ± S.E. –fold change in human hepatocytes compared with Fa2N-4 cells determined from three independent experiments, as described in Materials and Methods.

Pubmed ID	Gene	mRNA Expression Levels Relative to Fa2N-4			
		527	455	LHO	DMQ
NM_000499	CYP1A1	23.79±9.88*	11.71±1.90*	62.11±28.75*	15.83±3.88*
NM_000761	CYP1A2	19.26±6.08*	>100[c]*	97.67±0.74*	>100[c]*
NM_000762	CYP2A6	4.12±0.18*	0.70±0.34	0.42±0.24	48.11±18.00*
NM_000767	CYP2B6[a]	3.95±0.66*	2.87±0.84	2.70±0.57	10.24±0.66*
NM_000769	CYP2C19[b]	2.05±0.56	0.38±0.21*	1.03±0.04	2.07±0.33
NM_000770	CYP2C8	2.09±0.86	3.34±0.59*	2.70±1.00*	25.57±6.35*
NM_000771	CYP2C9[a]	1.05±0.46	0.66±0.10*	1.07±0.18	2.02±1.12
NM_000106	CYP2D6	5.33±1.01*	3.6±1.9*	6.05±0.14*	32.90±13.53*
NM_000773	CYP2E1	19.81±2.88*	45.78±15.03*	92.66±18.68*	>100[c]*
NM_017460	CYP3A4[a]	0.75±0.39	0.27±0.03*	18.19[b]	6.61±2.86
NM_022820	CYP3A43	0.64±0.71	32.01±6.22	3.34±4.47	2.60±2.26
NM_000777	CYP3A5[b]	0.56±0.15*	2.56±1.12*	3.58[d]	24.31±6.87*
NM_000765	CYP3A7	34.38±8.84*	n.d.	62.0±5.0	25.38[d]
NM_023944	CYP4F12	1.50±0.05	0.48±0.06*	1.43±0.15	2.28±1.40
NM_000846	GSTA2[a]	2.03±0.59*	0.46±0.05*	5.23±0.53*	9.08±2.48*
NM_000847	GSTA3	>100[c]*	0.70±0.09	6.12±2.65*	38.91±1.10*
NM_177536	SULT1A1	4.76±3.54	>100[cd]	44.37±72.77	0.94±0.17
NM_177528	SULT1A2	1.31±1.11	5.14±0.48*	29.01±23.84	6.63±0.62*
NM_024044	SULT1A3;GIYD2;GIYD1[a]	0.21±0.07*	0.11±0.06*	0.30±0.05*	0.64±0.12*
NM_014465	SULT1B1	0.05±0.04	3.26±2.44	0.07±0.03*	0.01±0.00*
NM_005420	SULT1E1[a]	0.02±0.01	0.00±0.00*	0.04±0.01*	0.07±0.04*
NM_003167	SULT2A1	0.77±0.21	0.27±0.04*	0.78±0.22	4.06±1.37*
NM_000367	TPMT	0.43±0.18*	0.11±0.04*	0.15±0.02*	0.31±0.08*
NM_000463	UGT1A1	1.25±0.06*	2.68±0.98*	1.38±0.26*	3.96±1.47*
NM_001072	UGT1A6[a,b]	26.79±5.61*	5.42±0.97*	19.33±1.98*	18.83±9.60*
NM_019076	UGT1A8	0.78±0.55	0.34±0.22*	0.47±0.19	0.94±0.47
NM_001076	UGT2B15	17.64±5.91*	13.3±7.0*	19.98±6.83*	>100[c]*
NM_021139	UGT2B4	60.95±31.57*	75.48±13.65*	>100[c]*	>100[c]*
NM_001074	UGT2B7	9.96±1.06*	1.42±0.11	1.67±0.57	18.14±2.60*

* $P < 0.05$, ΔC_t value significantly different from Fa2N-4 cells.

[a] – Fa2N-4 cells gene expression increased > 10-fold during the 48 hour adaptation period.

[b] – Cryopreserved human hepatocyte gene expression increased > 10-fold during the 24 hour adaptation period.

[c] - Low gene expression in Fa2N4 cells (Ct>34).

[d] – Data were expressed as the average value of experiments performed in duplicate.

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Table 2: Comparison of transcription factor gene expression between Fa2N-4 cells and cryopreserved human hepatocytes.

The gene expression levels were evaluated in human hepatocytes and Fa2N-4 cells after the 24h and 48h adaptation periods, respectively. Data are expressed as means ± S.E. –fold change in human hepatocytes compared with Fa2N-4 cells determined from three independent experiments, as described in Materials and Methods.

Pubmed ID	Gene	mRNA Expression Levels Relative to Fa2N-4			
		527	455	LHO	DMQ
NM_001621	A*h*R	0.21±0.09*	0.06±0.02*	0.17±0.06*	0.14±0.04*
NM_002015	FOXO1A	0.27±0.04*	0.02±0.04	0.18±0.02*	0.62±0.64
NM_178849	HNF4α[a]	0.50±0.07	0.12±0.02*	0.30±0.02*	0.92±0.14
NM_147223	NCOA1	0.56±0.15	0.29±0.13*	0.43±0.08	0.50±0.14
NM_006540	NCOA2[a]	0.71±0.04*	0.24±0.07*	0.56±0.14	0.54±0.13
NM_181659	NCOA3	0.40±0.02*	0.17±0.04*	0.42±0.05	0.31±0.08*
NM_006311	NCOR1[a]	0.28±0.13*	0.12±0.01*	0.25±0.06*	0.26±0.05*
NM_001077261	NCOR2	0.30±0.08*	0.10±0.01*	0.32±08*	0.32±0.12*
NM_021969	NROB2[a]	28.22±2.17*	14.35±6.33*	47.92±2.58*	95.50±13.56*
NM_007121	NR1H2[a]	0.32±0.05*	0.12±0.03*	0.35±0.06*	0.35±0.04*
NM_005123	NR1H4[a,b]	0.02±0.02	0.01±0.0*	0.06±0.07	0.08±0.10
NM_033013	NR1I2	0.71±0.22	0.32±0.11*	0.65±0.19	2.44±0.56
NM_005122	NR1I3	>100[c]*	>100[c]*	>100[c]*	93.76±43.56*
NM_001018074	NR3C1	0.36±0.04*	0.06±0.01*	0.31±0.04*	0.19±0.04*
NM_003489	NRIP1	0.27±0.06*	0.19±0.11*	0.31±0.24	0.39±0.26
NM_013261	PPARGC1A[a]	0.04±0.01*	0.01±0.01	0.06±0.03*	0.06±0.02*
NM_002957	RXRA	0.27±0.05*	0.17±0.05*	0.22±0.02*	0.61±0.15
NM_000545	TCF1[a]	0.24±0.08*	0.05±0.02*	0.18±0.03*	0.30±0.01*
NM_001017535	VDR	1.10±0.37	0.14±0.11	0.54±0.13*	0.33±0.15*

* $P < 0.05$, ΔC_t value significantly different from Fa2N-4 cells.

[a] – Fa2N-4 cells gene expression increased > 10-fold during the 48 hour adaptation period.

[b] – Cryopreserved human hepatocyte gene expression increased > 10-fold during the 24 hour adaptation period.

[c] - Low gene expression in Fa2N4 cells (Ct>34).

[d] – Data were expressed as the average value of experiments performed in duplicate.

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Table 3: Comparison of transporter gene expression between Fa2N-4 cells and cryopreserved human hepatocytes.

The gene expression levels were evaluated in human hepatocytes and Fa2N-4 cells after the 24h and 48h adaptation periods, respectively. Data are expressed as means ± S.E. –fold change in human hepatocytes compared with Fa2N-4 cells determined from three independent experiments, as described in Materials and Methods.

Pubmed ID	Gene	Basal Expression Levels Relative to Fa2N-4			
		527	455	LHO	DMQ
NM_000927	ABCB1[a,b]	0.46±0.09*	0.26±0.05*	0.42±0.12*	0.62±0.10*
NM_003742	ABCB11	>100[c]*	>100[c]*	93.3[d]	>100[c]*
NM_000443	ABCB4	0.34±0.10*	1.13±0.31	1.64±1.27	1.64±0.74
NM_004996	ABCC1	0.13±0.05*	0.03±0.00*	0.10±0.04*	0.05±0.03*
NM_000392	ABCC2	2.26±0.60*	1.72±0.45*	5.33±0.66*	5.26±0.42*
NM_003786	ABCC3[a]	1.11±0.13	0.40±0.04*	0.79±0.07*	2.44±0.09*
NM_005845	ABCC4	0.81±0.31	0.61±0.25	0.49±0.11*	0.63±0.11
NM_001171	ABCC6[a]	0.89±0.01*	0.33±0.11*	0.45±0.02*	1.98±0.37
NM_004827	ABCG2[a]	0.50±0.01*	0.22±0.08*	0.89±0.35	0.56±0.08
NM_003049	SLC10A1	>100[c]*	>100[c]*	>100[c]*	>100[c]*
NM_003057	SLC22A1	16.01±1.95*	7.89±3.36*	27.43±6.77*	>100[c]*
NM_153320	SLC22A7	1.83±0.62*	2.08±0.56*	1.03±0.49	16.33±2.47*
NM_134431	SLCO1A2	1.04±0.83	5.82±1.01*	34.87±24.52	22.88±19.73
NM_006446	SLCO1B1[b]	>100[c]*	>100[c]*	>100[c]*	>100[c]*
NM_019844	SLCO1B3	55.09±22.10*	>100[c]*	>100[c]*	>100[c]*
NM_007256	SLCO2B1	2.83±1.02*	1.10±0.30	2.59±0.48*	12.89±2.65*

* $P < 0.05$, ΔC_t value significantly different from Fa2N-4 cells.

[a] – Fa2N-4 cells gene expression increased > 10-fold during the 48 hour adaptation period.

[b] – Cryopreserved human hepatocyte gene expression increased > 10-fold during the 24 hour adaptation period.

[c] - Low gene expression in Fa2N4 cells (Ct>34).

[d] – Data were expressed as the average value of experiments performed in duplicate.

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Table 4: EC_{50} and E_{max} values for induction of CYP3A4 and CYP2B6 mRNA for compounds tested in Fa2N-4 cells and human hepatocytes.

Compound	Fa2N-4				Human Hepatocytes (DMQ)			
	CYP3A4		CYP2B6		CYP3A4		CYP2B6	
	EC_{50}(μM)	E_{max}(fold)	EC_{50}(μM)	E_{max}(fold)	EC_{50}(μM)	E_{max}(fold)	EC_{50}(μM)	E_{max}
Artemisinin[a]	nq	nq	nq	nq	21.4±1.8	29.4±1.7	40.6±28.1	12.4±4.0
CITCO[a]	nq	nq	nq	nq	0.8±0.7	5.3±4.9	0.01±0.004	6.8±0.6
Phenytoin[c]	10.0±1.2	9.2±0.8	nq	nq	5.1±3.1	16.9±0.2	9.8±2.0	10.7±1.5
Phenobarbital[c,e]	nq	nq	204.4±54.4	4.99±3.6	nq	nq	60.4±4.9	31.3±1.4
Rifampicin[b]	8.0±5.9	59.5±17.3	8.0±1.6	4.1±0.5	0.4±0.3	52.5±26.7	2.3±0.5	12.9±0.8
Moricizine[d]	1.6±0.5	7.1±0.5	nq	nq	1.3±0.2	17.2±0.8	0.05[f]	2.8±0.4
Bosentan[b]	1.8±0.7	11.5±1.2	nq	nq	1.0±0.1	47.7±3.3	0.4±0.1	3.8±0.3

EC_{50} (μM) and E_{max} (fold change) are expressed as mean ± calculated standard error (SE) using SigmaPlot Version 10 as described in Materials and Methods.
Studies conducted with Fa2N-4 cells represent the mean of three independent experiments performed in triplicate.
Studies conducted in human hepatocytes (DMQ) represent the mean of one experiment done in duplicate.
[a] – Compound is a CAR-activator (Maglich et al., 2003; Simonsson et al., 2006).
[b] – Compound is a PXR-activator (LeCluyse, 2001; Luo et al., 2002b; van Giersbergen et al., 2002).
[c] – Gene induction occurs via cross talk between PXR and CAR (Bell and Michalopoulos, 2006; Trubetskoy et al., 2005; Wang et al., 2004).
[d] – Compound is a PXR activator (unpublished data EC_{50} = 1.7±0.3 μM).
[e] – Dose-response curve did not reach saturation for CYP3A4 induction within concentrations tested.
[f] – High standard error.
nq- not quantifiable

Figure 1

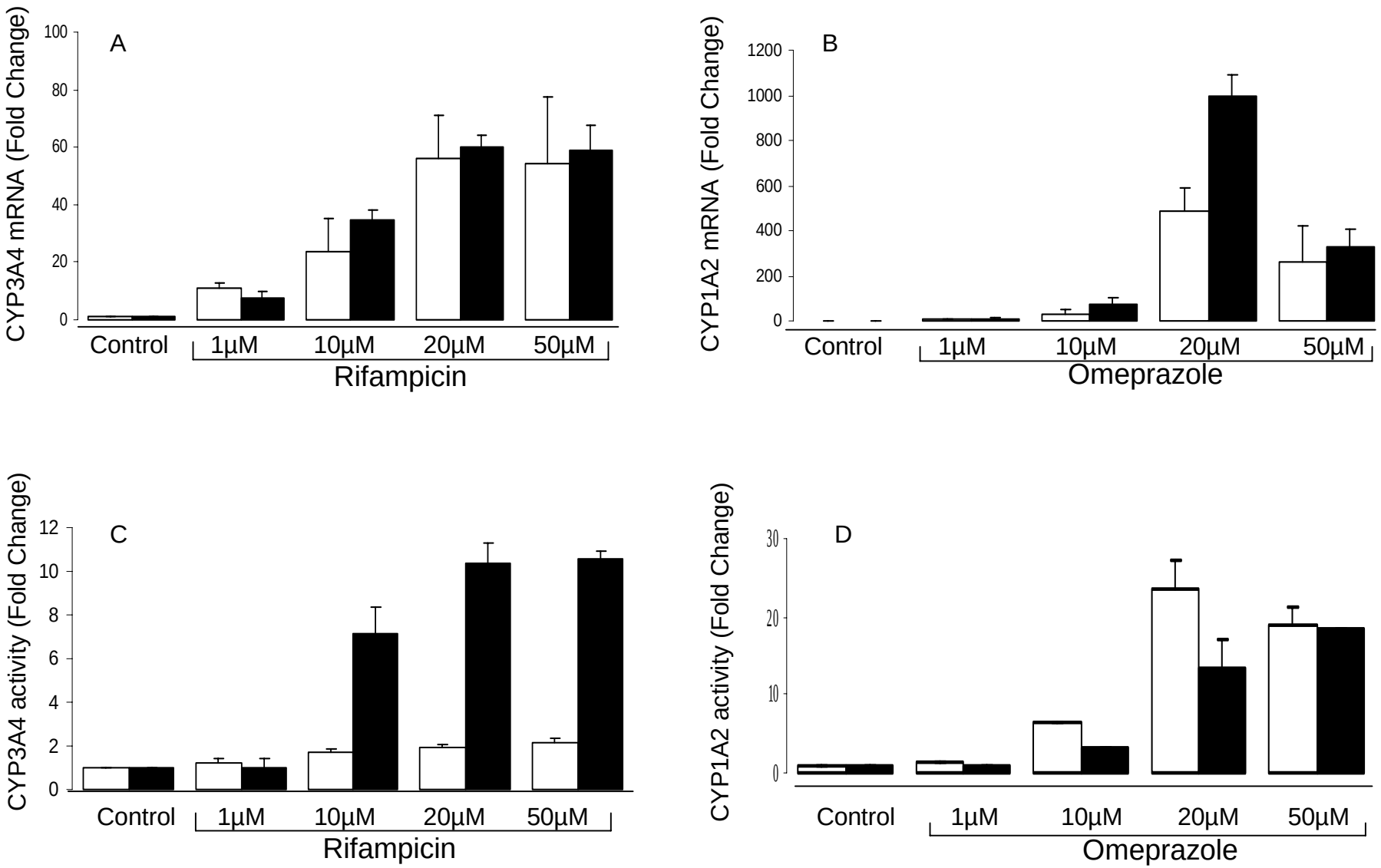

Figure 2

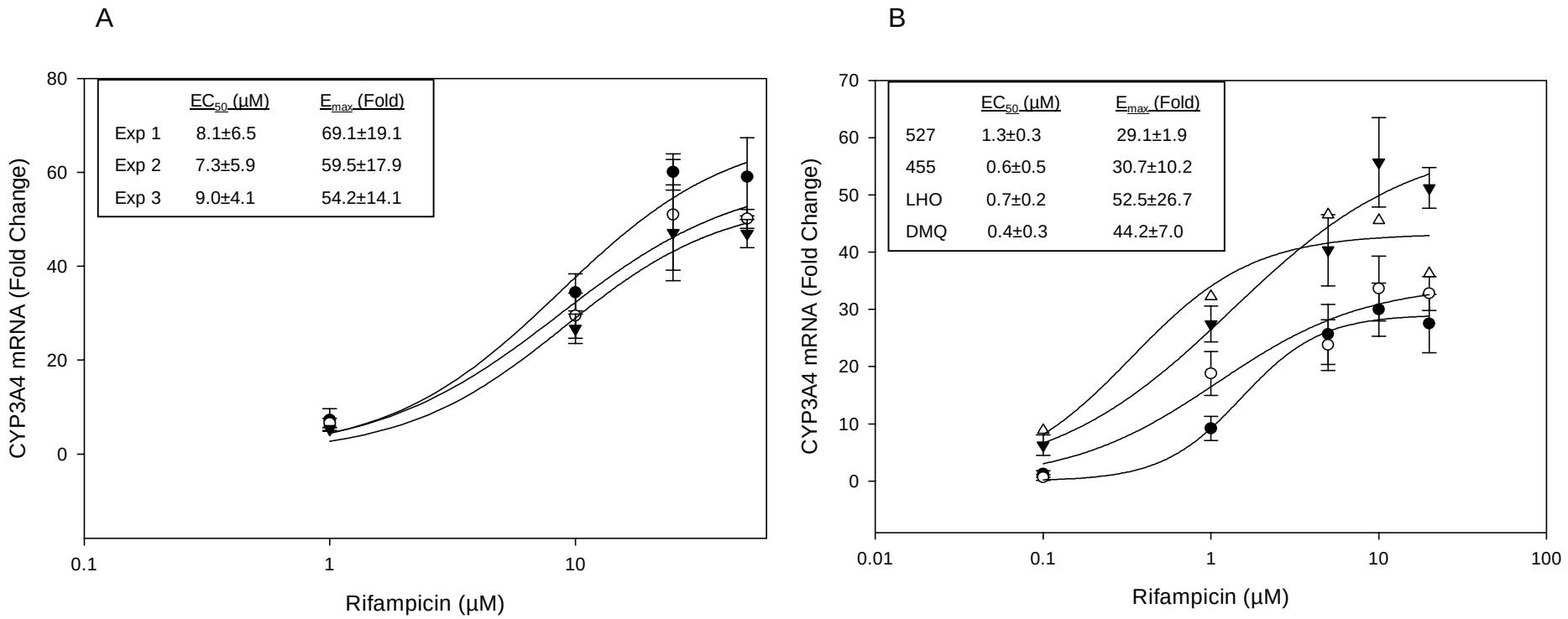

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Figure 3

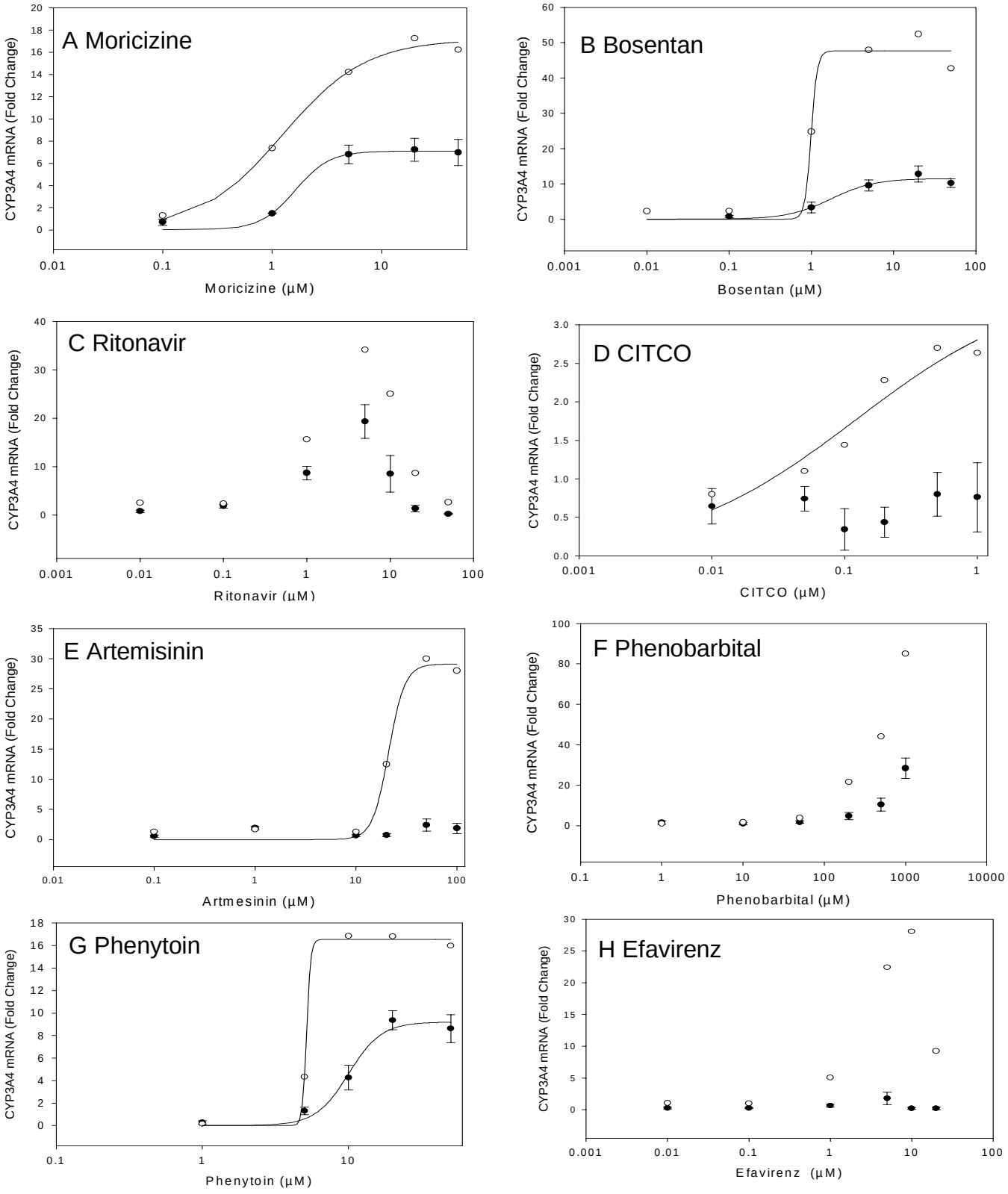

Figure 4