Activity, Inhibition, and Induction of Cytochrome P450 2J2 in Adult Human Primary Cardiomyocytes

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ABSTRACT

Cytochrome P450 2J2 plays a significant role in the epoxidation of arachidonic acid to signaling molecules important in cardiovascular events. CYP2J2 also contributes to drug metabolism and is responsible for the intestinal clearance of ebastine. However, the interaction between arachidonic acid metabolism and drug metabolism in cardiac tissue, the main expression site of CYP2J2, has not been examined. Here we investigate an adult-derived human primary cardiomyocyte cell line as a suitable model to study metabolic drug interactions (inhibition and induction) of CYP2J2 in cardiac tissue. The primary human cardiomyocyte cell line demonstrated similar mRNA-expression profiles of P450 enzymes to adult human ventricular tissue. CYP2J2 was the dominant isozyme with minor contributions from CYP2D6 and CYP2E1. Both terfenadine and astemizole oxidation were observed in this cell line, whereas midazolam was not metabolized suggesting lack of CYP3A activity. Compared with recombinant CYP2J2, terfenadine was hydroxylated in cardiomyocytes at a similar K_m value of 1.5 μM. The V_max of terfenadine hydroxylation in recombinant enzyme was found to be 29.4 pmol/pmol P450 per minute and in the cells 6.0 pmol/pmol P450 per minute. CYP2J2 activity in the cell line was inhibited by danazol, astemizole, and ketoconazole in submicromolar range, but also by xenobiotics known to cause cardiac adverse effects. Of the 14 compounds tested for CYP2J2 induction, only rosiglitazone increased mRNA expression, by 1.8-fold. This cell model can be a useful in vitro model to investigate the role of CYP2J2-mediated drug metabolism, arachidonic acid metabolism, and their association to drug induced cardiotoxicity.

Introduction

Cytochrome P450 2J2 has attracted particular attention for its ability to epoxidize arachidonic acid regioselectively to 5,6-, 8,9-, 11,12-, or 14,15-epoxyeicosatrienoic acids (EETs) (Roman, 2002). These EETs have many biological functions including, but not limited to, angiogenesis, regulation of vasodilation, inhibition of cytokine-induced endothelial cell adhesion-molecule expression, inhibition of vascular smooth muscle cell migration, protection of endothelial cells against hypoxia-reoxygenation injury, upregulation of endothelial nitric oxide biosynthesis, and protection of doxorubicin-induced cardiotoxicity (Larsen et al., 2007; Spector and Norris, 2007; Yang et al., 2009; Zhang et al., 2009; Campbell and Fleming, 2010; Pfister et al., 2010). All these events are involved in cardiac electrophysiology and protect the heart from ischemic-reperfusion injury (Spiecker and Liao, 2006). More specifically, the regioisomer 11,12-EET has been shown to be a potent activator of the ion channels sensitive to ATP, to directly decrease the membrane action potential in rat myocytes (Lu et al., 2001; Hashizume et al., 2002; Matsumoto et al., 2002; Liu et al., 2006; Lafite et al., 2007), anticancer drug tamoxifen, and drugs such as thioridazine or cyclopamine (Lee et al., 2012). The combination of cardiac localization and involvement in the arachidonic acid metabolism makes CYP2J2 a particularly interesting target to mechanistically investigate drug-induced cardiotoxicity.

CYP2J2 is predominantly expressed in extrahepatic tissues, particularly in the heart, but also in skeletal muscle, placenta, small intestine, kidney, lung, pancreas, bladder, and brain (Wu et al., 1997; Zeldin et al., 1997; Bieche et al., 2007). While a crystal structure has yet to be elucidated, molecular models suggest structural similarity between CYP2J2 and CYP3A4, explaining why the two enzymes share a number of substrates of diverse therapeutic areas, such as the antihistamine drugs terfenadine, astemizole, and ebastine (Matsumoto and Yamazoe, 2001; Hashizume et al., 2002; Matsumoto et al., 2002; Liu et al., 2006; Lafite et al., 2007), anticancer drug tamoxifen, and drugs such as thioridazine or cyclopamine (Lee et al., 2012). The combination of cardiac localization and involvement in the arachidonic acid metabolism makes CYP2J2 a particularly interesting target to mechanistically investigate drug-induced cardiotoxicity.

So far, no studies have demonstrated drug metabolism in the heart tissue. The inhibitory or inductive effect by such drugs on arachidonic acid metabolism could have profound downstream consequences by reducing EETs and their protective properties. However, a human heart model remains elusive and testing relies on animal-model, especially dog, cell systems or recombinant enzymes. Much of CYP2J2’s activity has been assessed in such models as Escherichia coli-expressed or Baculovirus-infected insect cell–expressed enzyme (Supersomes) (Lafite et al., 2007), human liver microsomes (Lee et al., 2012), or in humanized animal models that overexpress the enzyme in cardiac tissue (Seubert et al., 2004; Deng et al., 2011).

In this study, we evaluate commercially available primary human cardiomyocytes for expression and activity of CYP2J2. We first cloned...
and expressed CYP2J2 and measured its activity. Second, we evaluated the expression of a range of important P450s in addition to CYP2J2 in human cardiomyocytes by mRNA content compared with levels of P450 expression in human ventricular tissue. Third, we assessed the metabolic activity of CYP2J2 in the cardiomyocytes toward probe substrates and characterized the kinetic parameters compared with recombinantly expressed enzyme. Finally, we investigated the induction and inhibition of CYP2J2 in these cardiomyocytes by various compounds especially ones known to cause cardiotoxicity.

Materials and Methods

Chemicals and Cell Culture Materials. All chemicals including terfenadine and astemizole were purchased from Sigma-Aldrich (St. Louis, MO), unless otherwise stated, and used without further purification. Acetonitrile, methanol, water, ammonium formate, and formic acid were purchased from Fisher Scientific (Pittsburgh, PA). Adult-derived primary human cardiomyocytes, cell culture media (complete growth media and serum-free media), solutions, and cell culture materials (culture flasks and plates, precoated with proprietary matrix for cell adherence) were purchased from Celprogen Inc. (San Pedro, CA).

Cloning of the Expression Constructs. The CYP2J2 cDNA was a gift from Dr. Darryl Zeldin at the National Institute of Environmental and Health Sciences. An internal Ndel site in CYP2J2 was removed using the QuickChange II XL site-directed mutagenesis kit (Stratagene, La Jolla, CA) with primers 5'-GAAATTTGTTGTTCATCGATGGACAAACACAG, 3'-CTGGTGGTTCATATCATGGCTAGACGAGAAACACATTCT (Ndel site in italics, change from wild-type underlined), one unit of Pfu polymerase, and cycling conditions of 95°C for 3 minutes followed by 18 cycles of 94°C for 30 seconds, 55°C for 45 seconds, 68°C for 10 minutes. The resulting construct (CYP2J2-Ndel) was excised and inserted into the pCWori expression vector (Guryev et al., 2001) used as a template to generate the pCW2J2 expression construct (Barnes et al., 1991). The constructs were generated by PCR amplification with the primers 5'-ACTCATATTGGCTCTGTATAGCTAGTTCATCTTCAACAAAGCGGCCTC and the same reverse primer: ATTCATGTCGACCATGAGAAGACGGCGCACAGAGGGCTT, and a 2% agarose gel, excised with a scalpel, and recovered with the Qiaquick gel extraction kit and ligated overnight with 1 IU of T4 DNA ligase.

Protein Expression. Protein expression was performed as previously described (Cheesman et al., 2003; Kaspera et al., 2011) and harvested cells were resuspended in storage buffer and stored in −80°C until purification.

Protein Purification. Frozen pellets were thawed on ice and resuspended in 100 mM potassium phosphate (pH 7.4) containing 20% glycerol and protease inhibitors. Purification was conducted following established procedures (Kaspera et al., 2011).

Measurement of P450 Concentration. CO-difference spectra were obtained to determine the concentration of purified CYP2J2 according to the method of (Omura and Sato 1964).

Determination of Kinetic Parameters Km and Vmax. Enzyme activity was determined for recombinant enzymes at varying protein concentrations. Reactions were conducted for 3 minutes followed by 28 cycles of 94°C for 30 seconds, 55°C for 45 seconds, 68°C for 2 minutes. These primers incorporated an Ndel site into the 5' primer and a SalI site into the 3' primer and the pCWori plasmid contains a SalI site followed by a 6His tag to facilitate subsequent purification. The N-terminus was then truncated (MLAAMGSLAAALAWVHRPLTLLGTVAPLAAADFLKRSP to MARRR). The resulting amplification products and the pCWori plasmid were digested with Ndel and SalI, resolved on a 2% agarose gel, excised with a scalpel, and recovered with the Qiagen gel extraction kit.

Human Heart Tissue. Human heart tissue was harvested from the University of Washington Medical Center. Tissue from six individual donors (n = 6, 3 male, 3 female) undergoing transplant procedures were used in this study. Frozen human heart tissue was provided and used for all experiments in this study were of ventricular cardiac cells. All experiments were carried out with cells initiated from a cell stock frozen at passage four and cultured to passage six. Cells used for RNA work were detached by trypsin digestion, neutralized with media, harvested, and pelleted by centrifugation at 100g for 5 minutes. The pelleted tissue was washed with phosphate-buffered saline (PBS), and stored in 30 µl of RNAlater solution. Total RNA was extracted from approximately 1 million cells using the MagMax-96 Total RNA Isolation Kit (Life Technologies, Carlsbad, CA) and from human heart tissue using Trizol Reagent and PureLink RNA Mini Kit (Life Technologies). Total RNA was then used to synthesize cDNA using Oligo dT20 primers and the Superscript III First Strand Synthesis System (Life Technologies). Reverse-transcription polymerase chain reaction (RT-PCR) was performed using TaqMan (Life Technologies) FAM reporter primers for the various cytochrome P450s screened as well as the housekeeping gene GusB. Each biologic triplicate was performed in technical triplicates such that the values reported are an average of nine data points.

Culturing of human cardiomyocytes was established following Celprogen’s protocols. Cells were grown in an incubator set at 37°C with 5% CO2 atmosphere. The batch obtained and used for all experiments in this study were of ventricular cardiac cells. All experiments were carried out with cells initiated from a cell stock frozen at passage four and cultured to passage six. Cells used for RNA work were detached by trypsin digestion, neutralized with media, harvested, and pelleted by centrifugation at 100g for 5 minutes. The pelleted tissue was washed with phosphate-buffered saline (PBS), and stored in 30 µl of RNAlater solution. Total RNA was extracted from approximately 1 million cells using the MagMax-96 Total RNA Isolation Kit (Life Technologies, Carlsbad, CA) and from human heart tissue using Trizol Reagent and PureLink RNA Mini Kit (Life Technologies). Total RNA was then used to synthesize cDNA using Oligo dT20 primers and the Superscript III First Strand Synthesis System (Life Technologies). Reverse-transcription polymerase chain reaction (RT-PCR) was performed using TaqMan (Life Technologies) FAM reporter primers for the various cytochrome P450s screened as well as the housekeeping gene GusB. Each biologic triplicate was performed in technical triplicates such that the values reported are an average of nine data points.

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system. Ten microliters of the sample was injected on a Phenomenex (Torrance, CA) Aeria PEPTIDE XB-C8 column (1.7 µm, 150 × 2.10 mm). The mobile phases consisted of aqueous phase A: 0.1% formic acid in H2O, and organic phase B: 0.1% formic acid in acetonitrile. The samples were analyzed using the following gradient: mobile phase B: 0–3 minutes, 3%; 3–5 minutes, 3–10%; 5–8 minutes, 10–50%; 8–8.4 minutes, 50%; 8.4–8.5 minutes, 50–90%; 8.5–9.5 minutes, 90%; 9.5–10 minutes, 90–3%; 10–10.5 minutes, 3%. The column was re-equilibrated for 1 minute before the next injection.

To determine Km and Vmax of CYP2J2, incubations were carried out as outlined for Km and Vmax determination of CYP2J2. The reaction was quenched by addition of acetonitrile (100 µM) containing 0.1 µM midazolam as internal standard. Vigorous pipetting was then used to facilitate cellular detachment from the plate and lysis. The samples were centrifuged (3500 rpm) and 200 µl was transferred to a new 96-well plate for analysis.

Induction of CYP2J2 mRNA in Human Cardiomyocytes. Cells that had been plated in 6-well plates and allowed to attach overnight were treated with potential inducers: phenytoin (100 µM), phenobarbital (100 µM), dexamethasone (100 µM), rifampin (10 µM), clotrimazole (100 µM), omeprazole (100 µM), rosiglitazone (100 µM), ritonavir (10 µM), N-ethyl-N-(4-nitrophenyl)maleimide (500 µM), orrostestosterone was also tested at different concentrations (0.01, 0.1, 1, 10, and 100 µM). The cells were kept for 48 hours in media containing the inducing agent. Media was changed at 24 hours to replenish inducers. After 48 hours, the cells were detached, pelleted, and mRNA content was analyzed as mentioned above. mRNA was extracted from approximately 1 million cells.
Results

Expression and Kinetics of Recombinant *E. coli*-Expressed CYP2J2. SDS-PAGE analysis showed a band at 57 kDa consistent with full-length CYP2J2 protein, and a CO-difference spectrum showed active P450 and no inactive P420 present (data not shown). Expressed CYP2J2 protein was assayed for metabolic activity using terfenadine, which displayed Michaelis-Menten kinetics with a $K_m$ of 1.55 μM (Fig. 1, Table 1). Enzyme activity was expressed as rate of alcohol metabolite formed, using the peak height as a quantitative comparison with internal standard.

**Cytochrome P450 mRNA Screen.** CYP2J2 was the major isozyme expressed among the P450s that were screened in human cardiomyocytes (Fig. 2). CYP2D6 and CYP2E1 were also detected at levels approximately 20-fold below that of CYP2J2. In human heart tissue, CYP2J2 had also the greatest expression level. Several other P450 isozymes complemented CYP2J2 expression in human heart tissue, including CYP2C8, CYP2D6, CYP2E1, CYP2V1, CYP3A4, CYP4A11, CYP4B1, and CYP4F12, but expression levels were at least 50-fold lower than that of CYP2J2.

**CYP2J2 Protein Content Determination.** Using mass spectrometry for detection, the average expression of CYP2J2 in cardiomyocytes is 2.96 pmol per 1 million cells.

**Kinetic Parameters of Drug Metabolism in Human Cardiomyocytes.** Drug metabolic activity was measured in the cells using both terfenadine and astemizole as probe drugs. Both drugs were oxidized and exhibited Michaelis-Menten kinetics with a $K_m$ of 1.51 μM (Fig. 3A, Table 1) for terfenadine hydroxylation and $K_m$ of 5.22 μM for astemizole demethylation (Fig. 3B, Table 1). In contrast to astemizole, terfenadine was toxic to the cells at higher concentrations.

**Inhibition of CYP2J2 in Human Cardiomyocytes.** Inhibition was assessed at two concentrations of substrate [0.2 μM, Fig. 4A, and 1.5 μM (at $K_m$), Fig. 4B] and two concentrations of inhibitor (1 and 10 μM). Danazol and ketoconazole greatly inhibited the enzyme at both substrate concentrations. Danazol was equally potent at both concentrations of substrate, reducing activity about 95%, but ketoconazole was more potent at the lower substrate concentration. At 0.2 μM terfenadine (the $K_m$ for terfenadine hydroxylation found using Supersomes), astemizole, and cisapride also inhibited CYP2J2 at both inhibitor concentrations. Pimozide reduced activity by >60% at the higher inhibitor concentration of 10 μM and by approximately 15% at an inhibitor concentration of 1 μM. Other drugs tested exhibited little to no inhibition. Levomethadyl and sertindole appear to activate the enzyme by up to 50%. At 1.5 μM terfenadine, inhibition of CYP2J2 activity was reduced, with many drugs exhibiting little (as much as 20%) to no inhibition (Fig. 4A). Astemizole, cisapride, and pimozide still inhibited enzyme activity, as much as 60% in the case of 1 μM astemizole, but the degree to which they inhibited was not as pronounced as it was at substrate concentration of 0.2 μM (Fig. 4B).

**Hormone Effects on Gene Expression.** CYP2J2 induction by sex hormones β-estradiol and testosterone demonstrated that β-estradiol increased mRNA transcript levels in a concentration-dependent manner, while testosterone decreased transcription of CYP2J2 (Fig. 5). However, changes in the levels of transcription were not statistically different from control untreated cells.

**Induction of CYP2J2 in Human Cardiomyocytes.** Fig. 6, A and B presents the mRNA and activity following induction using the following drugs and concentrations: phenytoin (100 μM), phenobarbital (100 μM expression), 750 μM activity), dexamethasone (100 μM), rifampin (10 μM), clotrimazole (100 μM expression, 50 μM activity), omeprazole (100 μM), rosiglitazone (100 μM), ritonavir (10 μM), β-naphthoflavone (100 μM expression, 50 μM activity), butylated hydroxyanisole (100 μM), butylated hydroxytoluene (100 μM), and carbamazepine (100 μM).

When examining CYP2J2 mRNA expression, many of the compounds screened did not result in an increased gene expression (Fig. 6A). An increase in CYP2J2 mRNA was observed when the cells were treated

![Diagram of mRNA expression in human cardiomyocytes and human ventricular heart tissue.](https://example.com/figure2.png)
with rosiglitazone (≥50% increase), BHA (50% increase), and BHT (40% increase). Slight decreases in mRNA content were observed in the cells when treated with dexamethasone, clotrimazole, and ritonavir.

The greatest increase in enzyme activity occurred when the cells were treated with carbamazepine (30% increase), though this was not significant. Ritonavir treatment showed >95% decrease in terfenadine hydroxylation by CYP2J2. Phenytoin, phenobarbital, rosiglitazone, omeprazole, and clotrimazole also reduced CYP2J2 activity (Fig. 6B). Other compounds did not appreciably affect the enzyme’s ability to oxidize terfenadine.

Postinduction, there was no appreciable decrease in protein levels in cells treated with rosiglitazone, ritonavir, or BHT indicating that these agents do not affect protein stability. (Supplemental Fig. 1)

Intracellular levels of terfenadine postinduction were also measured. In cells treated with ritonavir and rosiglitazone, terfenadine levels were decreased by 50% compared with untreated cells but were unchanged relative to control when treated with BHT. (Supplemental Fig. 2)

Experiments to determine if rosiglitazone inhibited CYP2J2-mediated metabolism of terfenadine showed that rosiglitazone at 100 μM concentration does not inhibit CYP2J2 activity (data not shown).

Discussion

Here a primary cardiac cell line was examined for its potential use to screen for cardiac metabolism–related liabilities. These ventricular cells are derived from adult humans, which is important considering the interspecies differences in CYP2J activity previously reported (Ma et al., 2004; Yamasaki et al., 2004; Aiba et al., 2006; Elshenawy et al., 2013). Further, much of the drug-induced cardiotoxicity can be attributed to ventricular tissue. The P450 mRNA expression profile was similar to human cardiac ventricular tissue, with CYP2J2 by far the dominant isoform. The ability of the cells to metabolize CYP2J2 substrates astemizole and terfenadine was also established. Various compounds most notably danazol and ketoconazole readily inhibited CYP2J2 activity. However, CYP2J2 mRNA were mostly unchanged in the presence of potential inducers.

Others have shown the dominant presence of CYP2J2 in cardiac tissue, using immunoblotting or quantitative real-time PCR (Wu et al., 1996; Michaud et al., 2010). The expression of various P450 isozymes in the heart, including CYP1A1, CYP2B6, CYP2C8, CYP2C19, CYP2J2, and CYP2E1, are also reported (Wu et al., 1996; Thum and Borlak, 2000; Michaud et al., 2010). In the cardiac cell line, the expression of CYP2J2 agrees well with previously published data but the cellular expression levels of the CYP2C subfamily were below limits of detection. Delozier et al. (2007) detected CYP2C2 in cardiac tissue samples that were prepared from whole heart tissue. The cells investigated here are derived from ventricular tissue and do not contain endothelial cells. It is possible that the CYP2C expression in the heart tissue is localized to endothelial cells and not cardiomyocytes.

Fig. 3. Kinetic parameters of terfenadine hydroxylation (A) and astemizole demethylation (B) in human cardiomyocytes.

Fig. 4. Inhibition of terfenadine hydroxylation at 0.2 μM (A) and 1.5 μM (B) at 1-μM and 10-μM inhibitor concentrations after 2 hours of incubation in human cardiomyocytes.
Km values for terfenadine hydroxylation were comparable in the cells and E. coli-expressed system but were 10-fold higher than Supersomes (1.5 \mu M versus 0.2 \mu M, respectively). The similarity of terfenadine hydroxylation seen in cells and E. coli models (with deviations at high substrate concentration due to inhibition or cell toxicity) is a promising indication that these cells present a well suited model of drug metabolism in the heart. Similar protein content of 0.2-0.3 pmol CYP2J2 were used for Km experiments carried out using the cardiomyocytes and E. coli expressed recombinant protein. It should be noted that the E. coli expressed enzyme CYP2J2 has a truncation at the N-terminus and a 6xHis-tag at the C-terminus for purification purposes. It is unclear at this time whether these modifications alter the enzyme’s activity to any significant degree. Another potential source of variability is the difference in the ratio between CYP2J2 and its redox partners cytochrome P450 reductase and cytochrome b5. Supersome systems by BD Gentest have variable ratios, while reconstituted systems maintain a 1:2:1 ratio of CYP/CPR/b5. Further, commercial Supersomes contain human CPR, while reconstituted systems use rat CPR. In addition, the role of specific and nonspecific binding of terfenadine to the cells in altering the Km value cannot be determined at this time.

To test the inhibition of terfenadine hydroxylation in the heart, potential inhibitors with a documented history of cardiotoxicity were selected. Danazol was included because it is a specific inhibitor of CYP2J2 and causes congestive heart failure with prolonged use (Lee et al., 2012). Two inhibitor concentrations were used (1 and 10 \mu M) to resemble more closely plasma-level concentrations and accumulation due to inhibited metabolism or transport. Further, two concentrations of substrate (0.2 and 1.5 \mu M) were chosen to reflect the measured in vitro Km values for terfenadine in the different in vitro systems. Using substrate concentrations at sub-Km levels would reflect the competitive inhibition more clearly operating in the linear range of substrate turnover. As expected, danazol greatly inhibited CYP2J2 in this cell system, reinforcing CYP2J2’s role in metabolism of terfenadine in the heart. The inhibition of CYP2J2 activity by drugs such as ketoconazole and ritonavir were also expected, particularly because these drugs are reported to inhibit CYP2J2 in Supersomes, and are also known to inhibit CYP3A4 (Lee et al., 2012). Interestingly, sertindole, tacrolimus, and levomethadyl at lower concentrations increased CYP2J2 activity, possibly due to allosterism or other cell distribution phenomena (such as transport) not accounted for in this study.
Induction of CYP2J2 was evaluated at both the transcriptional and protein activity levels. A 48-hour induction period was chosen after preliminary studies indicated that significant cell death occurred at 72 hours. Lee and Murray (2010) reported BHA as a CYP2J2 inducer in HepG2 cells. Further work by Ma et al. (2004) has shown that the mouse ortholog CYP2J5 is regulated by sex hormones in murine kidneys. The results of this study, however, show that in cardiomyocyte, neither BHA nor the sex hormone β-estradiol affect the transcription of the CYP2J2. Testosterone had a slight repressive effect at high concentration indicating possible gender differences in regulation. Incubation of the cells with terfenadine immediately following inducer treatment does not appear to result in increased protein activity, suggesting an unlikely change in protein levels.

It is possible that CYP2J2 is differentially regulated in various cell types and different organs. It is important to note that Lee and Murray (2010) induced their cells with BHA for 72 hours compared with the 48 hours of this study. Further, they replenished the BHA in their cell media frequently during their induction (at 6, 12, 18, 24, and 48 hours), whereas BHA was replenished at 24 hours in this study. This inability to induce CYP2J2 in cardiomyocytes indicates an important endogenous function involving tightly regulated expression and activity to preserve or protect the cell. This is supported by the G-50T mutation, the only other notable CYP2J2-allele reported across ethnic groups. Carriers of this allele have decreased expression of the CYP2J2 gene and have been shown to have increased risk of adverse cardiac effects (Spiecker et al., 2004; Marciani et al., 2008; Zhang et al., 2008).

A delicate balance of expression levels might be needed, and interference with physiologic pathways could have detrimental effects. Other compounds tested for the ability to induce CYP2J2 transcription and CYP2J2 activity are classic P450 inducers, which bind to the pregnane X receptor (PXR) (Fahmi et al., 2012). Of note, rosiglitazone simultaneously induced transcription of mRNA but also inhibited terfenadine hydroxylation. Rosiglitazone is a known PXR inducer (Sinz et al., 2006); however, if rosiglitazone was operating through the PXR receptor, then rifampin should have induced mRNA as well. Rosiglitazone is potentially binding and inducing CYP2J2 through peroxisome proliferator-activated receptor (PPAR), which also induces mRNA of CYP2B and CYP4 enzymes (Rogue et al., 2010).

Also, while our goal was to find potential inducers of CYP2J2 transcription and CYP2J2 protein, it appears that some drugs reduced terfenadine hydroxylation, such as ritonavir and rosiglitazone. The decrease in terfenadine hydroxylation could potentially be due to the drug inhibiting the transporter responsible for uptake of terfenadine into the cell. Our data shows that the amount of terfenadine remaining in the cell was at least 50% lower than control samples (Supplemental Fig. 2). This indicates that terfenadine is perhaps unable to enter the cell following the induction treatment due to the inhibition of transporters by xenobiotics. Currently, not much is known about which drug following the induction treatment due to the inhibition of transporters (Mordvinink et al., 2013), making the primary cells investigated here a promising alternative.

In conclusion, this work provides an important step toward identifying a model that could investigate metabolism-related drug adverse effects in the heart during preclinical investigations. The cardiomyocyte cell line is of human-derived ventricular cells, but it is important to note that these primary lines exhibit potential drawbacks (e.g., heterogeneity of the donors, indefinite cultivation, donor age, drug use). Finding a model that is appropriate to all circumstances is difficult, but these primary human cardiomyocytes present a simpler applicable tool than in vivo studies and thus a promising avenue forward.


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Supplemental Figure 1. CYP2J2 protein content following a 48 h treatment with rosiglitazone, ritonavir and butylated hydroxytoluene. Values are normalized to an untreated sample set to 100%.
Supplemental Figure 2. Terfenadine content in treated samples, expressed as a percent of protein detected in untreated controls following 48 h treatment with rosiglitazone, ritonavir, and butylated hydroxytoluene.