Vascular Pharmacology xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Murine atrial HL-1 cell line is a reliable model to study drug metabolizing enzymes in the heart

Osama H. Elshenawy, Anwar Anwar-Mohamed, Ghada Abdelhamid, Ayman O.S. El-Kadi *

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2E1

ARTICLE INFO

Article history:
Received 24 August 2012
Received in revised form 23 November 2012
Accepted 15 December 2012
Available online xxxx

Keywords:
HL-1
Cytochrome P450s
Arachidonic acid metabolism
Epoxyeicosatrienoic acids
Dihydroxyeicosatrienoic acids
Epoxygenases
Hydroxylases
Cardiac hypertrophy

ABSTRACT

HL-1 cells are currently the only cells that spontaneously contract while maintaining a differentiated cardiac phenotype. Thus, our objective was to examine murine HL-1 cells as a new *in vitro* model to study drug metabolizing enzymes. We examined the expression of cytochrome P450s (Cyps), phase II enzymes, and nuclear receptors and compared their levels to mice hearts. Our results demonstrated that except for Cyp4a12 and Cyp4a14 all Cyps, phase II enzymes: glutathione-*S*-transferases (Gsts), heme oxygenase-1 (HO-1), and NAD(P)H: quinone oxidoreductase (Nqo1), nuclear receptors: aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane X receptor (PXR), and peroxisome proliferator activated receptor (PPAR-alpha) were all constitutively expressed in HL-1 cells. Cyp2b19, Cyp2c29, Cyp2c38, Cyp2c40, and Cyp4f16 mRNA levels were higher in HL-1 cells compared to mice hearts. Cyp2b9, Cyp2c44, Cyp2j9, Cyp2j11, Cyp2j13, Cyp4f13, Cyp4f15 mRNA levels were expressed to the same extent to that of mice hearts. Cyp1a1, Cyp1a2, Cyp1b1, Cyp2b10, Cyp2d10, Cyp2d22, Cyp2e1, Cyp2j5, Cyp2j6, Cyp3a11, Cyp4a10, and Cyp4f18 mRNA levels were lower in HL-1 cells compared to mice hearts. Moreover, 3-methylcholanthrene induced Cyp1a1 while fenofibrate induced Cyp2j9 and Cyp4f13 mRNA levels in HL-1 cells. Examining the metabolism of arachidonic acid (AA) by HL-1 cells, our results demonstrated that HL-1 cells metabolize AA to epoxyeicosatrienoic acids, dihydroxyeicosatrienoic acids, and 20-hydroxyeicosatetraenoic acids. In conclusion, HL-1 cells provide a valuable *in vitro* model to study the role of Cyps and their associated AA metabolites in addition to phase II enzymes in cardiovascular disease states.

1. Introduction

Cardiovascular diseases (CVD) are the leading cause of death worldwide; every 36 seconds 1 person dies from CVD in the United States. Moreover, CVD exceeds the next four leading causes of death (cancer, lung disease, accidents and diabetes) combined (DiPiro, 2008). Recently, it was reported that there is a strong correlation between arachidonic acid (AA) metabolism and the pathogenesis of many CVD (Roman, 2002). It has long been recognized that cyclooxygenase (COX) and lipoxygenase (LOX) enzymes metabolize AA; however, a third pathway has been identified for AA metabolism, this pathway is the cytochrome P450 (Cyp) pathway (Capdevila et al., 1981). The Cyp pathway is further divided into Cyp epoxygenases and Cyp hydroxylases subpathways (Roman, 2002), Cyp epoxygenases metabolize AA to form epoxyeicosatrienoic acids (EETs), while Cyp hydroxylases form hydroxyeicosatetraenoic acids (HETEs). Now it is well established that, both EETs and HETEs play critical roles in cardiovascular system (Roman, 2002); EETs produce important biological effects such as regulating vascular tone in the coronary, cerebral, mesenteric, renal, pulmonary, and peripheral circulations, besides its anti-inflammatory activities (Anwar-mohamed et al., 2010). In contrast to EETs, 20-HETE is involved in many cardiovascular diseases including cardiac hypertrophy and heart failure (Anwar-mohamed et al., 2010).

All studies examining the role of drug metabolizing enzymes in the heart use either *in vivo* models such as mice or rats (Imaoka et al., 2005; Zordoky et al., 2008), or *in vitro* systems such as isolated neonatal and adult cardiomyocytes (Lee et al., 2004; Thum and Borlak, 2000a) or immortalized cell lines such as H9C2 cells (Zordoky and El-Kadi, 2007). However, each model has its limitations and drawbacks. For example, *in vivo* experiments are expensive and require multiple approvals due to ethical concerns (Festing and Wilkinson, 2007). Isolation of adult cardiomyocytes is a difficult technique as heart muscle cells are firmly connected to each other and it is difficult to cleave these connections without injuring the cells (Schlüter and Piper, 2005). Neonatal cardiomyocytes lack many characteristics of adult cardiomyocytes and are usually overgrown by nonmyocytes after a few days in culture (White et al., 2004). Finally the H9C2 cell line was originally derived from embryonic rat ventricular tissue which is no longer able to beat (Watkins et al., 2011).

Therefore, there is an urgent need for a reliable *in vitro* cell line model to study the role of drug metabolizing enzymes in the heart. Currently, the murine HL-1 cell line, from C57BL/6 mouse, is the only established immortalized cardiac cells that continuously divide

* Corresponding author at: Faculty of Pharmacy & Pharmaceutical Sciences, 2142J Katz Group-Rexall Centre for Pharmacy and Health Research, University of Alberta, Edmonton, Alberta, Canada T6G 2E1. Tel.: +1 780 492 3071; fax: +1 780 492 1217.
E-mail address: aelkadi@pharmacy.ualberta.ca (A.O.S. El-Kadi).

1537-1891/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.vph.2012.12.002

and spontaneously contracts while maintaining a differentiated cardiac phenotype (Claycomb et al., 1998). However, to the best of our knowledge, there has been no previous attempt to investigate the expression of different Cyp isoenzymes in HL-1 cells. Therefore, we hypothesize that HL-1 cell line may express different Cyp isoenzymes and therefore could be a good *in vitro* model to study drug metabolizing enzymes and their role in cardiac diseases. Thus, the present study aims to investigate the expression of Cyp isoenzymes in HL-1 cells and correlate this expression to *in vivo* model.

2. Materials and methods

2.1. Cell culture

HL-1 cells were cultured as previously described (Andersen et al., 2009; Ikeda et al., 2009) with slight modification. Briefly, cells were plated in 6-well culture plates pre-coated with 0.02% Bacto gelatin (Becton, Dickinson, and Company, Franklin Lakes, NJ, USA) and 5 μg/ml fibronectin (Sigma–Aldrich Inc., St Louis, MO, USA) and cultured in Claycomb Medium (Sigma–Aldrich Inc.) supplemented with 10% fetal bovine serum (FBS, Sigma–Aldrich Inc.), 0.1 mM norepinephrine (Sigma–Aldrich Inc.), 2 mM L-Glutamine (Invitrogen Corp.), and antibiotics (100 Units/ml penicillin and 100 μg/ml streptomycin; Invitrogen Corp.). When cells are confluent the cell culture medium was replaced with serum free Dulbecco's modified Eagle's medium (Sigma–Aldrich Inc.) for 24 h, to avoid possible interference from Claycomb Medium components (Kim and Novak, 2007; White et al., 2004). Thereafter, the total RNA was isolated and Cyp, phase II metabolizing enzymes, nuclear receptors and hypertrophic markers mRNA expression was determined by reverse transcription–polymerase chain reaction (RT-PCR) followed by real-time PCR.

2.2. Cell treatments

To determine the inducibility of Cyp genes in HL-1 cells, cells were treated with 5 μM 3-methylcholanthrene (3-MC) for 6 h or 10 μM fenofibrate for 24 h. Thereafter, the total RNA was isolated and gene expressions of Cyp1a1 in case of 3-MC and Cyp2j9 and Cyp4f13 in case of fenofibrate were assessed using real-time PCR.

2.3. Animals

All experimental procedures involving animals were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee. Six male C57BL/6 mice weighing 20–25 g were obtained from Charles River Canada (St. Constant, QC, Canada). Mice were euthanized and heart tissues were excised and immediately frozen in liquid nitrogen and stored at −80 °C. Thereafter, the total RNA was isolated and Cyp, phase II metabolizing enzymes, nuclear receptors and hypertrophic markers mRNA expression was determined by reverse transcription–polymerase chain reaction (RT-PCR) followed by real-time PCR.

2.4. RNA extraction and cDNA synthesis

Total RNA from HL-1 cells or frozen tissues was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions and quantified by measuring the absorbance at 260 nm; RNA purity was determined by measuring the 260/280 ratio. Thereafter, first strand cDNA synthesis was performed by using the High-Capacity cDNA reverse transcription kit (Applied Biosystems) according to the manufacturer's instructions. Briefly, 1.5 μg of total RNA from each sample was added to a mix of 2.0 μl of 10× reverse transcriptase buffer, 0.8 μl of 25× dNTP mix (100 mM), 2.0 μl of 10× reverse transcriptase random primers, 1.0 μl of MultiScribe reverse transcriptase, and 4.2 μl of nuclease-free water. The final reaction mix was kept at 25 °C for 10 min, heated to 37 °C for 120 min, heated for 85 °C for 5 min, and finally cooled to 4 °C.

2.5. Quantification by real-time PCR

Relative quantitative analysis of specific mRNA expression was performed using real-time PCR by subjecting the resulting cDNA to PCR amplification using 96-well optical reaction plates in the ABI Prism 7500 System (Applied Biosystems). The 25-μl reaction mix contained 0.1 μl of 10 μM forward primer and 0.1 μl of 10 μM reverse primer (40 nM final concentration of each primer), 12.5 μl of SYBR Green Universal Mastermix, 11.05 μl of nuclease-free water, and 1.25 μl of cDNA sample. The primers used in the current study are listed in Table 1. Assay controls were incorporated onto the same plate, namely, no-template controls to test for the contamination of any assay reagents. After sealing the plate with an optical adhesive cover, the thermocycling conditions were initiated at 95 °C for 10 min, followed by 40 PCR cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. Melting curve (dissociation stage) was performed by the end of each cycle to ascertain the specificity of the primers and the purity of the final PCR product.

2.6. Real-time PCR data analysis

The real time-PCR data were analyzed using the relative gene expression i.e. ($\Delta\Delta C_T$) method as described in Applied Biosystems User Bulletin No.2 and explained further by Livak and Schmittgen (2001). Briefly, the ΔC_T values were calculated in every sample for each gene of interest as follows: $C_{T\ \text{gene of interest}} - C_{T\ \text{internal control gene}}$, with glyceraldehyde-3-phosphate dehydrogenase (Gapdh) as the internal control gene. Gapdh was expressed to same degree and in HL-1 cells and C57BL/6 mice hearts, i.e., same threshold cycle was found for both models using the same concentration of RNA (1.5 μg). Calculation of relative changes in the expression level of one specific gene ($\Delta\Delta C_T$) was performed by subtraction of ΔC_T of control from the ΔC_T of the corresponding groups. The values and ranges given in different figures were determined as follows: $2^{-\Delta\Delta CT} \pm$ S.E.M., where S.E.M. is the standard error of mean.

2.7. Metabolism of AA by HL-1 cells

To determine whether Cyp enzymes in HL-1 cells are metabolically active, cells were plated in 100 mm petri dishes and incubated with vehicle or with 50 μM AA for 3 h. AA metabolites were extracted from the media by ethyl acetate and dried using speed vacuum (Savant, Farmingdale, NY). Extracted AA and metabolites were analyzed using liquid chromatography–electrospray ionization mass spectrometry (LC–ESI–MS) (Waters Micromass ZQ 4000 spectrometer) method as described previously (Zordoky et al., 2010). Briefly, the mass spectrometer was operated in negative ionization mode with single ion recorder acquisition. The nebulizer gas was acquired from an in house high purity nitrogen source. The source temperature was set at 150 °C, and the voltages of the capillary and the cone were 3.51 KV and 25 V, respectively. The samples (10 μl) were separated on reverse phase C18 column (Kromasil, 250×3.2 mm) using linear gradient mobile phase system with a mobile phase of water/acetonitrile with 0.005% acetic acid at flow rate of 0.2 ml/min. The mobile phase system started at 60% acetonitrile, linearly increased to 80% acetonitrile in 30 min, increased to 100% acetonitrile in 5 min, and held for 5 min.

2.8. Statistical analysis

Data are presented as mean ± S.E.M. Control and treatment measurements were compared using Student's *t* test. A result was considered statistically significant where $p<0.05$.

Please cite this article as: Elshenawy, O.H., et al., Murine atrial HL-1 cell line is a reliable model to study drug metabolizing enzymes in the heart, Vascul. Pharmacol. (2013), http://dx.doi.org/10.1016/j.vph.2012.12.002

Table 1
Primers sequences used for RT-PCR reactions.

	Forward primer	Reverse primer
Cyp1a1	5′-GGTTAACCATGACCGGGAACT-3′	5′-TGCCCAAACCAAAGAGAGTGA-3′
Cyp1a2	5′-ACAACGAGGGACACCTCAC-3′	5′-GGGATCTCCCCAATGCAC-3′
Cyp1b1	5′-AAT GAG GAG TTC GGG CGC ACA-3′	5′-GGC GTG TGG AAT GGT GAC AGG-3′
Cyp2b9	5′-GCT GCA GCT CAG CTA GTT ATG C-3′	5′-GCC CAC TGG CAA AAA ATA TAC C-3′
Cyp2b10	5′-GGG AAC CTC TTG CAG ATG-3′	5′-CCC AGG TGC ACT GTG AA-3′
Cyp2b19	5′-CAC AAA GCC TTC CTC ACC GAT-3′	5′-ACA AGC AAG CAA CCC ACA CTC-3′
Cyp2c29	5′-TGG TCC ACC CAA AAG AAA TTG A-3′	5′-GCA GAG AGG CAA ATC CAT TCA-3′
Cyp2c38	5′-GCA TTA CTT TTA GCA ATG GAA ACA GT-3′	5′-CCA CAA GAC ACT GTG CTT CTT CTC-3′
Cyp2c40	5′-TCC GGT TTT TGA CAA GGT TTC TAC-3′	5′-TGC CCA AGT TCC TCA AGG TAT TC-3′
Cyp2c44	5′-CTT TTC AAC GAG CGA TTC CC-3′	5′-TGT TTC TCC TCC TCG ATC TTG C-3′
Cyp2d10	5′-TCCACTGAATTTGCCACGC-3′	5′-TCAGCACGGAGGACATGTTG-3′
Cyp2d22	5′-CCACGCTTCATCAGGCTACTG-3′	5′-CACATTCAGGAACATGGGTAGGA-3′
Cyp2e1	5′-CCC AAG TCT TTA ACC AAG TTG GC-3′	5′-CTT CCA TGT GGG TCC ATT ATT GA-3′
Cyp2j5	5′-TGT GAA TCG CTT TAT GAC ACC G-3′	5′-TGA TGG GTC TCC TCC TGA ATG-3′
Cyp2j6	5′-TTAGCCACGATCTGGGCAG-3′	5′-CTGGGGGATAGTTCTTGGGG-3′
Cyp2j9	5′-GGG AAT GTT CTA AGC CTG GAT TT-3′	5′-GAG TGA CTG GGC GAT TCA TAA A-3′
Cyp2j11	5′-GTA TGA TGG ACA GTC ACC GGG A-3′	5′-GGT CCA GAG CAG TGC AGA TGA-3′
Cyp2j13	5′-GGG AAG AGG AAG GAC AGC CTT-3′	5′-GCA GCA GCT CCT GAA ACT GAC T-3′
Cyp3a11	5′-AGCAGGGATGGACCTGG-3′	5′-CGGTAGAGGAGCACCAA-3′
Cyp4a10	5′-GTG CTG AGG TGG ACA CAT TCA T-3′	5′-TGT GGC CAG AGC ATA GAA GAT C-3′
Cyp4a12	5′-TGA CCC CAG CTT TCC ACT ATG-3′	5′-TTG TTC AGG TCC TCA ACT GCC-3′
Cyp4a14	5′-GTC TCT CGG GGA GCA ATA TAC G-3′	5′-ACC AAT CCA GGG AGC AAA GAA-3′
Cyp4f13	5′-CCC TAA ACC GAG CTG GTT CTG-3′	5′-GAG TCG CAG GAT TGG GTA CAC-3′
Cyp4f15	5′-CCT GTG CTG TGT TCC TTA GGG-3′	5′-GAC GGG AAA TGA CCG TGA CT-3′
Cyp4f16	5′-CCG CCT CAG TTG TTT CCC TC-3′	5′-TGC CCA AGT GAC CTG AAA ACC-3′
Cyp4f18	5′-CTG CAT CCT CCC GTC ACT G-3′	5′-GGA TTG TGA TGT GTC CCG AAA-3′
Gsta1	5′-CCC CTT TCC CTC TGC TGA AG-3′	5′-TGC AGC TTC ACT GAA TCT TGA AAG-3′
Gsta2	5′-CCCCTTTCCCTCTGCTGAAG-3′	5′-TGCAGCCACACTAAAACTTGAAAA-3′
Gsta3	5′-TGGACAACTTCCCTCTCCTGAA-3′	5′-AATCTTCTTTGCTGACTCAACACAT-3′
Gsta4	5′-AACTTGTATGGGAAGGACCTGAA-3′	5′-CCACGGCAATCATCATCATC-3′
HO-1	5′-GTG ATG GAG CGT CCA CAG C-3′	5′-TGG TGG CCT CCT TCA AGG-3′
Nqo1	5′-GGA AGC TGC AGA CCT GGT GA-3′	5′-CCT TTC AGA ATG GCT GGC A-3′
AhR	5′-CGGCTTCTTGCAAAACACAGT-3′	5′-GTAAATGCTCTCGTCCTTCTTCATC-3′
CAR	5′-TCAACACGTTTATGGTGCAA-3′	5′-CTGCGTCCTCCATCTTGTAG-3′
PXR	5′-AAGAAGCAGACTCTGCCTTGGA-3′	5′-GTGGTAGCCATTGGCCTTGT-3′
PPAR-alpha	5′-GCAGCCTCAGCCAAGTTGAA-3′	5′-GCCCGGACAGCTTCCTAAGT-3′
ANP	5′-GGAGCCTACGAAGATCCAGC-3′	5′-TCCAATCCTGTCAATCCTACCC-3′
BNP	5′-AGTCCTTCGGTCTCAAGGCA-3′	5′-CCGATCCGGTCTATCTTGTGC-3′

3. Results

3.1. Expression level of Cyps, phase II metabolizing enzymes, nuclear receptors, and cardiac hypertrophic markers mRNA levels in HL-1 cells and C57BL/6 mice hearts

In the present study we showed that Cyp1, 2, 3 and 4 families are constitutively expressed in HL-1 cells as well as in C57BL/6 mice hearts. Levels of expression were determined based on criteria mentioned in Table 2. Cyp2d22, Cyp2j6, Cyp2j9, Cyp2j13, Cyp4f13, and Cyp4f16 were found to be highly expressed in both HL-1 cells and C57BL/6 mice hearts. Cyp2c29 was highly expressed in HL-1 cells, whereas, Cyp1a1, Cyp2b10, Cyp2e1, Cyp2j5, Cyp4a10, and Cyp4f18 were highly expressed in C57BL/6 mice hearts. Cyp1b1, Cyp2c44, Cyp2j11, Cyp3a11, and Cyp4f15 were found to be moderately expressed in both HL-1 cells and C57BL/6 mice hearts. Cyp2b19, Cyp2c38, Cyp2c40, Cyp2e1, Cyp2j5, Cyp4a10, and Cyp4f18 were moderately expressed in HL-1 cells, whereas, Cyp1a2, Cyp2c29, Cyp2d10, Cyp4a12, and Cyp4a14 were moderately expressed in C57BL/6 mice hearts. Low mRNA levels of Cyp1a1, Cyp1a2, Cyp2b10, Cyp2b9, and Cyp2d10 were found in HL-1, whereas, low mRNA levels of Cyp2b19, Cyp2b9, Cyp2c38, and Cyp2c40 were found in C57BL/6 mice hearts. Of interest, Cyp4a12 and Cyp4a14 were not detected in HL-1 cells compared to C57BL/6 mice hearts (data summarized in Table 3).

Phase II metabolizing enzymes including glutathione S-transferases (Gsta1, Gsta2, Gsta3, Gsta4), heme oxygenase 1 (HO-1), NAD(P)H quinone oxidoreductase 1 (Nqo1) in addition to the nuclear receptors; aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane X receptor (PXR) and peroxisome proliferator-activated receptor alpha (PPAR-alpha) were moderately to highly expressed in both HL-1 cells and C57BL/6 mice hearts. With regard to the cardiac

Table 2
Criteria for levels of expression of different genes.

Level	Threshold cycle
Highly expressed	<30
Moderately expressed	≥30 up to 35
Low expressed	≥35

Table 3
Common highly to moderately expressed Cyps, phase II metabolizing enzymes, nuclear receptors, and cardiac hypertrophic markers in both HL-1 cells and C57BL/6 mice hearts.

Cyps		Phase II metabolizing enzymes	Nuclear receptors	Cardiac hypertrophic markers
Cyp1b1	Cyp2j6	Gsta1	AhR	ANP
Cyp2c29	Cyp2j9	Gsta2	CAR	BNP
Cyp2c44	Cyp3a11	Gsta3	PXR	
Cyp2d22	Cyp4a10	Gsta4	PPAR-alpha	
Cyp2e1	Cyp4f13	HO-1		
Cyp2j11	Cyp4f15	Nqo1		
Cyp2j13	Cyp4f16			
Cyp2j5	Cyp4f18			

Please cite this article as: Elshenawy, O.H., et al., Murine atrial HL-1 cell line is a reliable model to study drug metabolizing enzymes in the heart, Vascul. Pharmacol. (2013), http://dx.doi.org/10.1016/j.vph.2012.12.002

hypertrophic markers ANP and BNP, they were highly expressed in both HL-1 cells and C57BL/6 mice hearts (data summarized in Table 3).

3.2. Constitutive expression of Cyps mRNA in HL-1 cells and C57BL/6 mice hearts

To examine the constitutive expression of various Cyps in HL-1 cells and C57BL/6 mice hearts, total RNA was isolated from untreated HL-1 cells and untreated C57BL/6 mice hearts, and different genes were determined by real-time PCR. Cyp2b9 and Cyp2b19 were the lowest expressed genes in HL-1 cells and C57BL/6 mice hearts, respectively, and thus were considered as calibrators.

Analysis of mRNA expression in C57BL/6 mice hearts revealed that the order of expression in family 1 was from highest to lowest as following: Cyp1a1 > Cyp1b1 > Cyp1a2 whereas in HL-1 cells it was Cyp1b1 > Cyp1a1 > Cyp1a2. Similarly for Cyp2 family the order of expression in C57BL/6 mice hearts was: Cyp2d22 > Cyp2j6 > Cyp2j9 > Cyp2e1 > Cyp2j13 > Cyp2b10 > Cyp2j5 > Cyp2j11 > Cyp2d10 > Cyp2c29 > Cyp2c44 > Cyp2c38 > Cyp2b9 > Cyp2c40 > Cyp2b19; whereas in HL-1 cells the order was: Cyp2j9 > Cyp2d22 > Cyp2j13 > Cyp2j6 > Cyp2c29 > Cyp2j11 > Cyp2c44 > Cyp2c38 > Cyp2j5 > Cyp2b19 > Cyp2e1 > Cyp2c40 > Cyp2d10 > Cyp2b10 > Cyp2b9. Lastly for Cyp4 family the order of expression in C57BL/6 mice hearts was: Cyp4f13 > Cyp4f16 > Cyp4f18 > Cyp4a10 > Cyp4a14 > Cyp4a12 > Cyp4f15; whereas in HL-1 cells the order was: Cyp4f16 > Cyp4f13 > Cyp4f18 > Cyp4a10 > Cyp4f15 (Fig. 1A and B).

3.3. Constitutive expression of phase II metabolizing enzymes, and nuclear receptors mRNA in HL-1 cells and C57BL/6 mice hearts

With regard to phase II metabolizing enzymes, Gsta2 was the lowest expressed gene in both HL-1 cells and C57BL/6 mice hearts, and thus was considered as a calibrator for phase II metabolizing enzymes relative gene expression. In C57BL/6 mice hearts the order of expression was from highest to lowest: Gsta4 > Nqo1 > HO-1 > Gsta3 > Gsta1 > Gsta2; whereas in HL-1 cells the order was: HO-1 > Nqo1 > Gsta4 > Gsta3 > Gsta1 > Gsta2 (Fig. 2A and B).

As for the nuclear receptors, AhR and PXR were the lowest expressed genes in HL-1 cells and C57BL/6 mice hearts, respectively, and thus were considered as calibrators for nuclear receptors. In C57BL/6, the order of expression was from highest to lowest: AhR > CAR > PPAR-alpha > PXR; whereas in HL-1 cells the order was PPAR-alpha > CAR > PXR > AhR (Fig. 2C and D).

3.4. Fold expression of Cyps mRNA in HL-1 cells relative to C57BL/6 mice hearts

All Cyp1 family enzymes were found to be expressed in C57BL/6 mice hearts and HL-1 cells. However Cyp1a1, Cyp1a2, and Cyp1b1 mRNA levels were lower in HL-1 cells by 99.98%, 90%, 91%, respectively, compared to C57BL/6 mice hearts (Fig. 3A). Regarding Cyp2 family, Cyp2b10 mRNA level was lower in HL-1cells by 99.8%, whereas, Cyp2b9 was almost expressed to the same mRNA level in HL-1cells compared to C57BL/6 mice hearts. Cyp2b19, Cyp2c29, Cyp2c38, Cyp2c40, and Cyp2c44 were higher in HL-1 cells by 609%, 506%, 536%, 305%, and 28%, respectively, compared to C57BL/6 mice hearts (Fig. 3B).

Cyp2d10, Cyp2d22, Cyp2e1, Cyp2j5, Cyp2j6, and Cyp2j11 mRNA levels were lower in HL-1cells by 98%, 81%, 99.7%, 98%, 86%, and 40%, respectively, compared to C57BL/6 mice hearts (Fig. 3C). Cyp2j9 and Cyp2j13 mRNA levels were expressed to the same level in HL-1cells compared to C57BL/6 mice hearts (Fig. 3C). With regard to Cyp3 and Cyp4 families, Cyp3a11 mRNA level was lower in HL-1 cells by 64% compared to C57BL/6 mice hearts; Cyp4a12 and Cyp4a14 were only

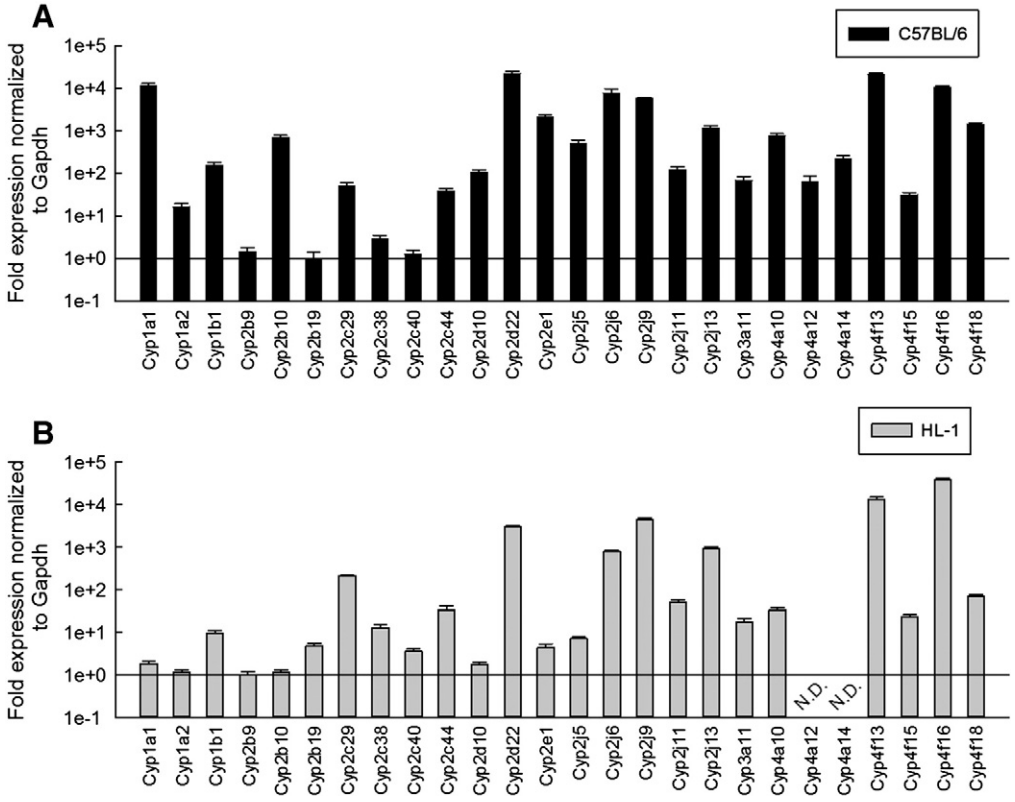

Fig. 1. Constitutive expression of different Cyp families mRNA in C57BL/6 mice hearts (A), and HL-1 cells (B). Total RNA was isolated from C57BL/6 mice hearts and HL-1 cells, and the relative expression of Cyp mRNA was determined by reverse transcription followed by real-time PCR. The data were analyzed using the relative gene expression method. The data were normalized to Gapdh and relative to a calibrator. Cyp2b19 and Cyp2b9 were the lowest expressed genes in C57BL/6 mice hearts and HL-1 cells, respectively, and thus were considered as calibrators. Results are presented as mean fold expression ± S.E.M. ($n = 6$).

Please cite this article as: Elshenawy, O.H., et al., Murine atrial HL-1 cell line is a reliable model to study drug metabolizing enzymes in the heart, Vascul. Pharmacol. (2013), http://dx.doi.org/10.1016/j.vph.2012.12.002

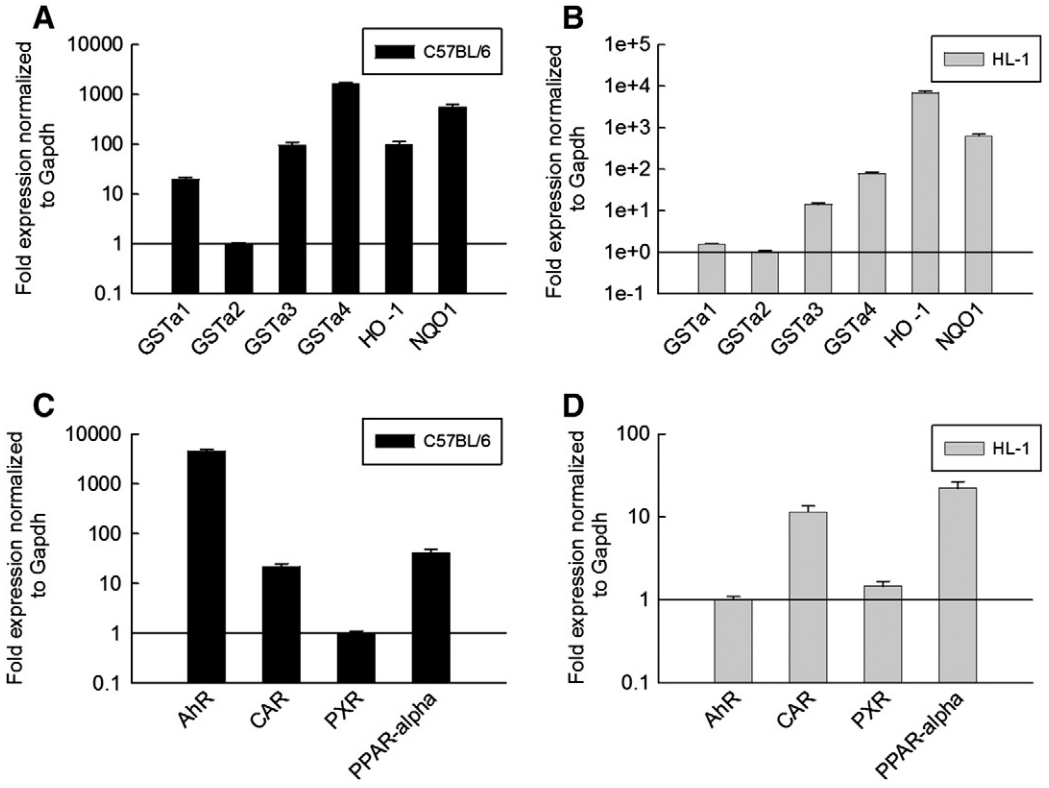

Fig. 2. Constitutive mRNA expression of phase two metabolizing enzymes in C57BL/6 mice hearts (A), phase two metabolizing enzymes in HL-1 cells (B), nuclear receptors in C57BL/6 mice hearts (C) and nuclear receptors in HL-1 cells (D). Total RNA was isolated from C57BL/6 mice hearts and HL-1 cells, and the relative expression was determined by reverse transcription followed by real-time PCR. The data were analyzed using the relative gene expression method. The data were normalized to Gapdh and relative to a calibrator. Gsta2 was the lowest expressed gene in both HL-1 cells and C57BL/6 mice hearts, and thus was considered as a calibrator for phase II metabolizing enzymes. PXR and AhR were the lowest expressed genes in C57BL/6 mice hearts and HL-1 cells, respectively, and thus were considered as calibrators for nuclear receptors. Results are presented as mean fold expression ± S.E.M. ($n = 6$).

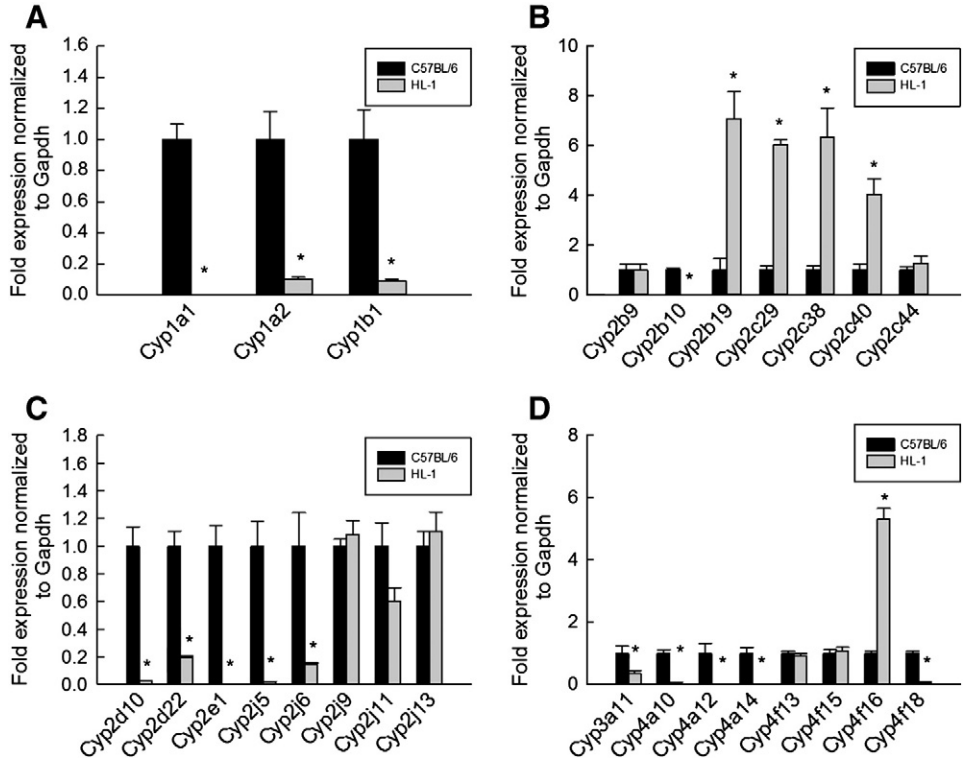

Fig. 3. Fold expression of Cyp1 family (A), Cyp2b and Cyp2c (B), Cyp2d, Cyp2e and Cyp 2j (C) and Cyp3 and Cyp4 families (D) in HL-1 cells relative to C57BL/6 mice hearts. Total RNA was isolated from C57BL/6 mice hearts and HL-1 cells, and the relative expression was determined by reverse transcription followed by real-time PCR. The data were normalized to Gapdh. Results are presented as mean fold expression ± S.E.M for HL-1 relative to C57BL/6. ($n = 6$). *, $p < 0.05$ compared with control.

Please cite this article as: Elshenawy, O.H., et al., Murine atrial HL-1 cell line is a reliable model to study drug metabolizing enzymes in the heart, Vascul. Pharmacol. (2013), http://dx.doi.org/10.1016/j.vph.2012.12.002

expressed in C57BL/6 mice hearts; Cyp4a10, Cyp4f13 and Cyp4f18 mRNA levels were lower in HL-1 cells by 94%, 10% and 93% respectively, compared to C57BL/6 mice hearts. Cyp4f15 mRNA level in HL-1cells was the same compared to C57BL/6 mice hearts. Cyp4f16 mRNA level in HL-1 cells was higher by 432% compared to C57BL/6 mice hearts (Fig. 3D).

3.5. Fold expression of phase II metabolizing enzymes, nuclear receptors, and cardiac hypertrophic markers in HL-1 cells relative to C57BL/6 mice hearts

Gsta1, Gsta2, Gsta3, Gsta4 and Nqo1 mRNA levels were lower in HL-1 cells by 99.6%, 95%, 99%, 99.8%, and 94% compared to C57BL/6 mice hearts; whereas, HO-1 was higher in HL-1 cells by 253% compared to C57BL/6 mice hearts (Fig. 4A). AhR, CAR and PPAR-alpha mRNA levels were lower in HL-1 cells by 99.98%, 42% and 42.2% compared to C57BL/6 mice hearts, while PXR was found to be higher in HL-1 cells by 60% compared to C57BL/6 mice hearts (Fig. 4B). ANP and BNP mRNA levels were lower in HL-1 cells by 68% and 98% respectively, compared to C57BL/6 mice hearts (Fig. 4C).

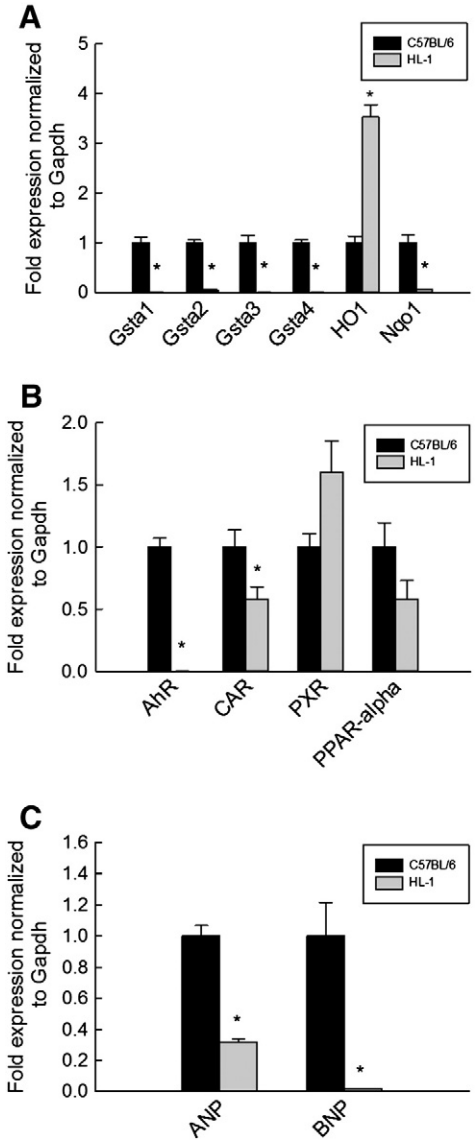

Fig. 4. Fold expression of nuclear receptors (A), phase two metabolizing enzymes (B), and hypertrophic markers (C) in HL-1 cells relative to C57BL/6 mice hearts. Total RNA was isolated from C57BL/6 mice hearts and HL-1 cells, and the relative expression was determined by reverse transcription followed by real-time PCR. The data were normalized to Gapdh. Results are presented as mean fold expression ± S.E.M for HL-1 relative to C57BL/6. ($n=6$). *, $p<0.05$ compared with control.

3.6. Effect of 3-MC and fenofibrate on Cyp gene expression

To examine the effect of Cyp inducers on the expression of various Cyp families, the cells were treated with 3-MC for 6 h or fenofibrate for 24 h. Thereafter, the expression of Cyp1a1 in case of 3-MC and Cyp2j9 and Cyp4f13 in case of fenofibrate was measured using real-time PCR. With regard to Cyp1 family, 3-MC induced Cyp1a1 by 94% as compared to control (Fig. 5). As for Cyp2 and Cyp4 families, fenofibrate induced Cyp2j9 and Cyp4f13 by 58% and 54%, respectively (Fig. 5).

3.7. Metabolic activity of Cyp epoxygenases and hydroxylases in HL-1

To examine the activity of Cyp enzymes in HL-1, cells were incubated with 50 μM AA for 3 h. Thereafter, AA metabolites were measured using LC–ESI–MS. Fig. 6A shows that HL-1 cells were able to metabolize AA into EETs namely 14,15-EET, 11,12-EET and 8,9-EET in the amounts of 77, 20, and 23 pmol/10^6 cells. However, 5,6-EET was below the detection level. DHETs namely 14,15-DHET, 11,12-DHET, 8,9-DHET and 5,6-DHET were formed in the amounts of 10, 19, 16, and 17 pmol/10^6 cells, respectively (Fig. 6B). In addition to 20-HETE which was formed in the amount of 10 pmol/10^6 cells (Fig. 6C).

4. Discussion

HL-1 cell line was employed to determine whether it will serve as a reliable *in vitro* model to study the drug metabolizing enzymes in the heart. Biotransformation of endogenous and exogenous substances is performed via phases I and phase II metabolizing enzymes (Nebert and Dalton, 2006). In general, transcriptional activation of most Cyps occurs through four main nuclear receptor mechanisms: the AhR for Cyp1 family, the CAR for the Cyp2 family; the PXR for the Cyp3 family; and PPAR for Cyp4 family (Aleksunes and Klaassen, 2012; Xu et al., 2005). In addition, AhR regulates some of phase II enzymes such as, Nqo1 and Gsta1 (Nebert and Duffy, 1997). Although liver is the main site for metabolism, most extrahepatic tissues express metabolizing enzymes to varying degrees. Recent studies proved that heart is not an exception, where different *in vivo* and *in vitro* models revealed the expression of different levels of Cyp isoenzymes (Michaud et al., 2010; Thum and Borlak, 2000b).

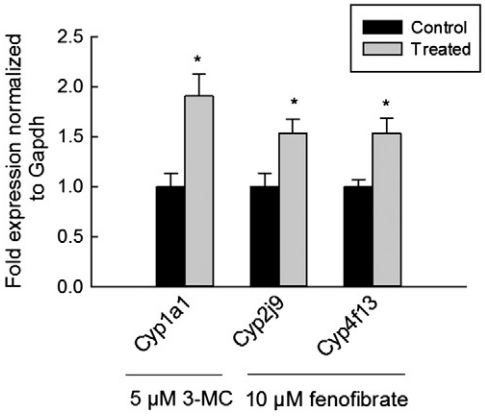

Fig. 5. Effect of 3-MC and fenofibrate on Cyp gene expression. Total RNA was isolated from HL-1 cells treated with vehicle, 5 μM 3-MC (Cyp1 inducer), or 10 μM fenofibrate (Cyp2 and 4 inducer). Thereafter, the relative expression was determined by reverse transcription followed by real-time PCR. The data were normalized to Gapdh. Results are presented as mean fold expression ± S.E.M. ($n=6$). *, $p<0.05$ compared with control.

Please cite this article as: Elshenawy, O.H., et al., Murine atrial HL-1 cell line is a reliable model to study drug metabolizing enzymes in the heart, Vascul. Pharmacol. (2013), http://dx.doi.org/10.1016/j.vph.2012.12.002

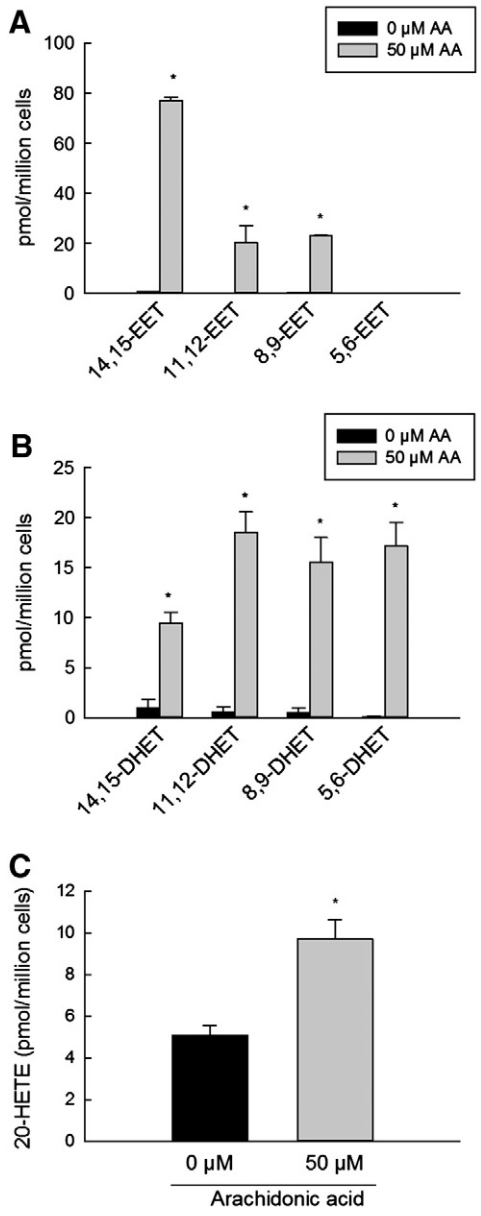

Fig. 6. Metabolic activity of Cyp epoxygenases and hydroxylases in HL-1. HL-1 cells were incubated with vehicle or 50 μM arachidonic acid for 3 h, thereafter EETs, DHETs, 20-HETE were extracted from the media by ethyl acetate and dried using speed vacuum. Reconstituted metabolites were injected into LC–ESI–MS for metabolite determination. Results are presented as mean ± S.E.M. ($n=6$). *, $p<0.05$ compared with control.

Cyp-mediated metabolites were recognized for their role in health and disease states of the heart (Elbekai and El-Kadi, 2006). Cyp epoxygenases and hydroxylases metabolize AA into EETs and HETEs participating in regulation of vascular tone, inflammatory and fibrotic properties. EETs serve as endothelium-derived hyperpolarizing factor (EDHF) providing a cardioprotective effect, while 20-HETE is a potent vasoconstrictor and considered as a cardiotoxic metabolite (Elbekai and El-Kadi, 2006). Phase II metabolizing enzymes such as Nqo1 and Gst are known to protect against oxidative stress and subsequent cardiovascular tissue injury (Dhalla et al., 2000; Idriss et al., 2008; Kim and Nel, 2005). In addition, HO-1 is the first and rate-controlling enzymatic step in heme degradation into ferrous (Fe^{2+}) iron, carbon monoxide (CO), and biliverdin which subsequently converted into bilirubin. These products are supposed to have physiological and pathological functions, including protection from oxidative stress and several cardiovascular diseases (Idriss et al., 2008).

Previous studies used different models to study the role of Cyp-mediated AA metabolism in the pathogenesis of heart diseases (Zordoky and El-Kadi, 2007). However, as illustrated previously, each of these models is associated with certain limitations and drawbacks; therefore, we investigated the expression of different Cyps mRNA in HL-1 cells as a new proposed model. We investigated the most important Cyp genes in xenobiotic metabolism that have been reported previously in the heart and/or isolated cardiomyocytes (Roman, 2002).

Low expression of Cyp1 family, Gsta1, and Nqo1 in HL-1 cells compared to C57BL/6 mice hearts was associated with low expression of AhR in HL-1 cells compared to C57BL/6 mice hearts. AhR is known to be one of the most important regulators for Cyp1 family, Gsta1, and Nqo1 (Aleksunes and Klaassen, 2012; Nebert and Duffy, 1997). With regards to Cyp2, Cyp3 and Cyp4 enzymes, we found that Cyp1b1, Cyp2c29, Cyp2c44, Cyp2d22, Cyp2e1, Cyp2j11, Cyp2j13, Cyp2j5, Cyp2j6, Cyp2j9, Cyp3a11, Cyp4a10, Cyp4f13, Cyp4f15, Cyp4f16, and Cyp4f18 were moderately to highly expressed in both HL-1 cells and C57BL/6 mice hearts. This finding was consistent with the expression of nuclear receptors CAR, PXR, and PPAR-alpha, known to regulate these families of Cyp isoenzymes (Aleksunes and Klaassen, 2012; Xu et al., 2005). All nuclear receptors investigated were moderately to highly expressed in both HL-1 cells and C57BL/6 mice hearts.

The difference observed in expression pattern of some Cyp isoenzymes could be attributed to three main factors. First, transcription factors such as AhR, CAR, PXR, and PPAR are essential for gene regulation but they lack the enzymatic activities necessary for modulating chromatin structure (Pascussi et al., 2008). These activities are catalyzed by co-regulators which are classified as co-activators and co-repressors (Pascussi et al., 2008). Second, it is known that transcription regulation is a complex process and usually more than one nuclear receptor is involved in the regulation of different Cyp isoenzymes (Anakk et al., 2004; Palut et al., 2002). Lastly, HL-1 is an immortalized cell line derived from adult C57BL/6J mouse (Claycomb et al., 1998), being immortalized cells provides a rationale for the cell line not to be exactly the same as normal tissues.

Recently, an interest emerged in HL-1 cells as a model to study cardiac hypertrophy being congruous and cost effective model (Brunt et al., 2009). We found that ANP and BNP levels in HL-1 cells are lower than C57BL/6 mice hearts; however, these hypertrophic markers are still highly expressed in HL-1 cells, based on threshold cycles, supporting their use as a model in mechanistic studies investigating cardiac hypertrophy.

In the current study we demonstrated that the Cyp enzymes are inducible in HL-1 cells by using 3-MC, which is known to induce Cyp1 family (Aboutabl et al., 2009; Elbekai et al., 2004) and fenofibrate, which is known to induce Cyp2 and Cyp4 families (Huang et al., 2007; Muller et al., 2004; Wilson et al., 1998). In addition, we demonstrated that HL-1 cells were able to convert AA to its biologically active metabolites 14,15-EET, 11,12-EET, 8,9-EET, 14,15-DHET, 11,12-DHET, 8,9-DHET, 5,6-DHET in addition to 20-HETE. Only 5,6-EET was not detected in control samples or in those incubated with AA. In fact 5,6-EET has two difficulties precluding an accurate estimate of its formation. First, 5,6-EET is unstable in physiological buffer. Second, 5,6-EET spontaneously degrades to the diol and lactone (Fulton et al., 1998). Interestingly, similar pattern for EETs and DHETs formation was previously observed *in vivo* with C57BL/6 mice, in which 14,15-EET was the abundant EET and 5,6-EET was the lowest detected EET, whereas all DHETs were produced to comparable levels (Anwar-Mohamed et al., 2012).

In conclusion, our findings provide the first evidence for the expression of different Cyp isoenzymes in HL-1 cells; moreover, Cyp epoxygenases (Cyp2b19, Cyp2c29, Cyp2c38, and Cyp2c40) and Cyp hydroxylases (Cyp2j9, Cyp4f15, and Cyp4f16) were expressed in HL-1 cells at same or higher levels compared to C57BL/6 mice hearts. In addition, Cyp enzymes in HL-1 are inducible and metabolically active. Therefore, this cell line offers a valuable *in vitro* model to study

Please cite this article as: Elshenawy, O.H., et al., Murine atrial HL-1 cell line is a reliable model to study drug metabolizing enzymes in the heart, Vascul. Pharmacol. (2013), http://dx.doi.org/10.1016/j.vph.2012.12.002

the pathophysiological role of Cyp epoxygenases and Cyp hydroxylases in the heart.

Acknowledgments

This work was supported by a grant from the Canadian Institutes of Health Research [Grant 106665] to A.O.S.E. O.H.E. is the recipient of University of Alberta Doctoral Recruitment Scholarship. A.A-M. is the recipient of Alberta Innovates Technology Futures Scholarship and Izaak Walton Killam Memorial Graduate Scholarship. We are grateful to Dr. John M. Seubert and Dr. Victor Samokhvalov for helping us with HL-1 cell culture.

References

Aboutabl, M.E., Zordoky, B.N., El-Kadi, A.O., 2009. 3-Methylcholanthrene and benzo(a)pyrene modulate cardiac cytochrome P450 gene expression and arachidonic acid metabolism in male Sprague Dawley rats. Br. J. Pharmacol. 158, 1808–1819.

Aleksunes, L.M., Klaassen, C.D., 2012. Coordinated regulation of hepatic phase I and II drug-metabolizing genes and transporters using AhR-, CAR-, PXR-, PPARalpha-, and Nrf2-null mice. Drug Metab. Dispos. 40, 1366–1379.

Anakk, S., Kalsotra, A., Kikuta, Y., Huang, W., Zhang, J., Staudinger, J.L., Moore, D.D., Strobel, H.W., 2004. CAR/PXR provide directives for Cyp3a41 gene regulation differently from Cyp3a11. Pharmacogenomics J. 4, 91–101.

Andersen, A.D., Poulsen, K.A., Lambert, I.H., Pedersen, S.F., 2009. HL-1 mouse cardiomyocyte injury and death after simulated ischemia and reperfusion: roles of pH, Ca2+−independent phospholipase A2, and Na+/H+ exchange. Am. J. Physiol. Cell Physiol. 296, C1227–C1242.

Anwar-mohamed, A., Zordoky, B.N., Aboutabl, M.E., El-Kadi, A.O., 2010. Alteration of cardiac cytochrome P450-mediated arachidonic acid metabolism in response to lipopolysaccharide-induced acute systemic inflammation. Pharmacol. Res. 61, 410–418.

Anwar-Mohamed, A., El-Sherbeni, A.A., Kim, S.H., Althurwi, H.N., Zordoky, B.N., El-Kadi, A.O., 2012. Acute arsenic toxicity alters cytochrome P450 and soluble epoxide hydrolase and their associated arachidonic acid metabolism in C57Bl/6 mouse heart. Xenobiotica 42, 1235–1247.

Brunt, K.R., Tsuji, M.R., Lai, J.H., Kinobe, R.T., Durante, W., Claycomb, W.C., Ward, C.A., Melo, L.G., 2009. Heme oxygenase-1 inhibits pro-oxidant induced hypertrophy in HL-1 cardiomyocytes. Exp. Biol. Med. (Maywood) 234, 582–594.

Capdevila, J., Chacos, N., Werringloer, J., Prough, R.A., Estabrook, R.W., 1981. Liver microsomal cytochrome P-450 and the oxidative metabolism of arachidonic acid. Proc. Natl. Acad. Sci. U. S. A. 78, 5362–5366.

Claycomb, W.C., Lanson Jr., N.A., Stallworth, B.S., Egeland, D.B., Delcarpio, J.B., Bahinski, A., Izzo Jr., N.J., 1998. HL-1 cells: a cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte. Proc. Natl. Acad. Sci. U. S. A. 95, 2979–2984.

Dhalla, N.S., Temsah, R.M., Netticadan, T., 2000. Role of oxidative stress in cardiovascular diseases. J. Hypertens. 18, 655–673.

DiPiro, J.T., 2008. In: DiPiro, Joseph T. (Ed.), Pharmacotherapy: A Pathophysiologic Approach, 7th edition. Content Technologies, United States.

Elbekai, R.H., El-Kadi, A.O., 2006. Cytochrome P450 enzymes: central players in cardiovascular health and disease. Pharmacol. Ther. 112, 564–587.

Elbekai, R.H., Korashy, H.M., Wills, K., Gharavi, N., El-Kadi, A.O., 2004. Benzo[a]pyrene, 3-methylcholanthrene and beta-naphthoflavone induce oxidative stress in hepatoma hepa 1c1c7 Cells by an AHR-dependent pathway. Free Radic. Res. 38, 1191–1200.

Festing, S., Wilkinson, R., 2007. The ethics of animal research. EMBO Rep. 8, 526–530.

Fulton, D., Falck, J.R., McGiff, J.C., Carroll, M.A., Quilley, J., 1998. A method for the determination of 5,6-EET using the lactone as an intermediate in the formation of the diol. J. Lipid Res. 39, 1713–1721.

Huang, H., Morisseau, C., Wang, J., Yang, T., Falck, J.R., Hammock, B.D., Wang, M.H., 2007. Increasing or stabilizing renal epoxyeicosatrienoic acid production attenuates abnormal renal function and hypertension in obese rats. Am. J. Physiol. Renal Physiol. 293, F342–F349.

Idriss, N.K., Blann, A.D., Lip, G.Y., 2008. Hemoxygenase-1 in cardiovascular disease. J. Am. Coll. Cardiol. 52, 971–978.

Ikeda, K., Tojo, K., Inada, Y., Takada, Y., Sakamoto, M., Lam, M., Claycomb, W.C., Tajima, N., 2009. Regulation of urocortin I and its related peptide urocortin II by inflammatory and oxidative stresses in HL-1 cardiomyocytes. J. Mol. Endocrinol. 42, 479–489.

Imaoka, S., Hashizume, T., Funae, Y., 2005. Localization of rat cytochrome P450 in various tissues and comparison of arachidonic acid metabolism by rat P450 with that by human P450 orthologs. Drug Metab. Pharmacokinet. 20, 478–484.

Kim, H.J., Nel, A.E., 2005. The role of phase II antioxidant enzymes in protecting memory T cells from spontaneous apoptosis in young and old mice. J. Immunol. 175, 2948–2959.

Kim, S.K., Novak, R.F., 2007. The role of intracellular signaling in insulin-mediated regulation of drug metabolizing enzyme gene and protein expression. Pharmacol. Ther. 113, 88–120.

Lee, S.J., Landon, C.S., Nazian, S.J., Dietz, J.R., 2004. Cytochrome P-450 metabolites in endothelin-stimulated cardiac hormone secretion. Am. J. Physiol. Regul. Integr. Comp. Physiol. 286, R888–R893.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) Method. Methods 25, 402–408.

Michaud, V., Frappier, M., Dumas, M.C., Turgeon, J., 2010. Metabolic activity and mRNA levels of human cardiac CYP450s involved in drug metabolism. PLoS One 5, e15666.

Muller, D.N., Theuer, J., Shagdarsuren, E., Kaergel, E., Honeck, H., Park, J.K., Markovic, M., Barbosa-Sicard, E., Dechend, R., Wellner, M., Kirsch, T., Fiebeler, A., Rothe, M., Haller, H., Luft, F.C., Schunck, W.H., 2004. A peroxisome proliferator-activated receptor-alpha activator induces renal CYP2C23 activity and protects from angiotensin II-induced renal injury. Am. J. Pathol. 164, 521–532.

Nebert, D.W., Dalton, T.P., 2006. The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. Nat. Rev. Cancer 6, 947–960.

Nebert, D.W., Duffy, J.J., 1997. How knockout mouse lines will be used to study the role of drug-metabolizing enzymes and their receptors during reproduction and development, and in environmental toxicity, cancer, and oxidative stress. Biochem. Pharmacol. 53, 249–254.

Palut, D., Kostka, G., Strucinski, P., 2002. The role of nuclear receptors in cytochrome P-450 induction by xenochemicals. Rocz. Panstw. Zakl. Hig. 53, 321–332.

Pascussi, J.M., Gerbal-Chaloin, S., Duret, C., Daujat-Chavanieu, M., Vilarem, M.J., Maurel, P., 2008. The tangle of nuclear receptors that controls xenobiotic metabolism and transport: crosstalk and consequences. Annu. Rev. Pharmacol. Toxicol. 48, 1–32.

Roman, R.J., 2002. P-450 metabolites of arachidonic acid in the control of cardiovascular function. Physiol. Rev. 82, 131–185.

Schlüter, K.-D., Piper, H., 2005. Isolation and culture of adult ventricular cardiomyocytes. In: Dhein, S., Mohr, F., Delmar, M. (Eds.), Practical Methods in Cardiovascular Research. Springer, Berlin Heidelberg, pp. 557–567.

Thum, T., Borlak, J., 2000a. Cytochrome P450 mono-oxygenase gene expression and protein activity in cultures of adult cardiomyocytes of the rat. Br. J. Pharmacol. 130, 1745–1752.

Thum, T., Borlak, J., 2000b. Gene expression in distinct regions of the heart. Lancet 355, 979–983.

Watkins, S., Borthwick, G., Arthur, H., 2011. The H9C2 cell line and primary neonatal cardiomyocyte cells show similar hypertrophic responses in vitro. In Vitro Cell. Dev. Biol. Anim. 47, 125–131.

White, S.M., Constantin, P.E., Claycomb, W.C., 2004. Cardiac physiology at the cellular level: use of cultured HL-1 cardiomyocytes for studies of cardiac muscle cell structure and function. Am. J. Physiol. Heart Circ. Physiol. 286, H823–H829.

Wilson, T.W., Alonso-Galicia, M., Roman, R.J., 1998. Effects of lipid-lowering agents in the Dahl salt-sensitive rat. Hypertension 31, 225–231.

Xu, C., Li, C.Y., Kong, A.N., 2005. Induction of phase I, II and III drug metabolism/transport by xenobiotics. Arch. Pharm. Res. 28, 249–268.

Zordoky, B.N., El-Kadi, A.O., 2007. H9c2 cell line is a valuable in vitro model to study the drug metabolizing enzymes in the heart. J. Pharmacol. Toxicol. Methods 56, 317–322.

Zordoky, B.N., Aboutabl, M.E., El-Kadi, A.O., 2008. Modulation of cytochrome P450 gene expression and arachidonic acid metabolism during isoproterenol-induced cardiac hypertrophy in rats. Drug Metab. Dispos. 36, 2277–2286.

Zordoky, B.N., Anwar-Mohamed, A., Aboutabl, M.E., El-Kadi, A.O., 2010. Acute doxorubicin cardiotoxicity alters cardiac cytochrome P450 expression and arachidonic acid metabolism in rats. Toxicol. Appl. Pharmacol. 242, 38–46.

Please cite this article as: Elshenawy, O.H., et al., Murine atrial HL-1 cell line is a reliable model to study drug metabolizing enzymes in the heart, Vascul. Pharmacol. (2013), http://dx.doi.org/10.1016/j.vph.2012.12.002