Short Communication

Characterization of THLE-Cytochrome P450 (P450) Cell Lines: Gene Expression Background and Relationship to P450-Enzyme Activity

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ABSTRACT:
The hepatic SV40 large T-antigen immortalized human liver epithelial (THLE) cell line and sublines transfected with cytochromes P450 (P450s) are increasingly being used for evaluation of potential drug-induced liver injury. So far, the available information on transporter and enzyme expression in these transfected cell systems is scattered. The purpose of this study was to characterize THLE cell lines with respect to transporter and enzyme expression. The mRNA expression of 96 typical drug metabolizing enzymes, and nuclear hormone receptors, was investigated in five THLE cell lines transfected with individual human P450s and in mock-transfected THLE-null cells using real-time polymerase chain reaction. The majority of the analyzed genes was either absent or expressed at low levels in the THLE-null and THLE-P450 cells, apart from housekeeping genes and the individual transfected P450s. Enzyme activity measurements provided confirmatory functional data for CYP2C9 and CYP3A4. Comparison with gene expression in human liver revealed an overall much lower gene expression in the THLE cell lines. The low levels of expression of a broad range of P450 genes in the THLE cell lines highlight the value of studies undertaken with P450-expressing cell lines for investigation of mechanisms of P450 metabolite-mediated hepatotoxicity. However, when attempting to translate between data obtained in THLE cell lines in vitro and functional consequences in vivo, it is important to take account of their limited expression of genes encoding many other drug-metabolizing enzymes and hepatic transporters.

Introduction

Drug-induced liver injury (DILI) is a leading cause of clinically significant adverse drug reactions (which include fatal liver failure), withdrawal of licensed drugs, failure to register new drugs, and compound termination due to toxicity during drug development (Kaplowitz, 2005). The causes for DILI are manifold and include both drug-related properties and characteristics of individual patients, which include genotype, underlying disease, comediations, and various other demographic factors (Pachkoria et al., 2007). Human hepatocytes have clear potential value in toxicity evaluations, because they express a broad range of metabolizing enzymes and other desirable differentiated functions (McGinnity et al., 2004; Hewitt et al., 2007). However, because use of hepatocytes to support assessment of toxicity during drug discovery is limited by their relatively high cost and restricted availability, alternative model systems are needed (Kalugkar and Soglia, 2005). One promising model is the THLE-P450 cell lines, which were developed by transfection of SV40 large T-antigen immortalized human liver epithelial cells with individual human cytochrome P450 (P450) isoforms CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 (Pfeifer et al., 1993). The panel of THLE cell lines have been used to explore involvement of individual human P450s in drug metabolism, in vitro gene toxicity, and in vitro cytoxicity (Antoine et al., 2008; Greer et al., 2010; Thompson et al., 2012).

The gene expression and/or enzymatic activities of some phase I and phase II enzymes has been reported to be low in THLE cells (Pfeifer et al., 1993; Dambach et al., 2005). However, a systematic analysis of expressed liver-typical enzymes and drug transporters within these cells is lacking. In particular, DILI in vivo is critically dependent upon the activities of plasma membrane transporters that mediate uptake and efflux of drugs (Hewitt et al., 2007; Giacomini et al., 2010), and currently the transporter capability of THLE-P450 cell lines is undefined. The purpose of this study was to investigate the gene expression background of five THLE-P450 cell lines, in direct comparison with human liver and the corresponding THLE-null cells. mRNA expression levels for a panel of 96 “liver typical” absorption, distribution, metabolism and excretion (ADME) genes were analyzed using reverse transcription-polymerase chain reaction (RT-PCR). In addition, in the THLE-2C9 and 3A4 cell lines, the P450 enzymatic activity was determined and compared with that in cryopreserved human hepatocytes.

ABBREVIATIONS: DILI, drug-induced liver injury; THLE, SV40 large T-antigen immortalized human liver epithelial; P450, cytochrome P450; ADME, absorption, distribution, metabolism and excretion; RT-PCR, reverse transcription-polymerase chain reaction; CT, threshold cycle; HPRT, hypoxanthine phosphoribosyl-transferase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PPIA, peptidylprolyl isomerase A; SLC, solute carriers; LXR, liver X receptor; PPAR, peroxisome proliferator-activated receptor; SULT, sulfotransferase; GST, glutathione-S-transferase.
**Materials and Methods**

**Cell Lines and Liver Tissue.** THLE cells (CYP1A2, -2C9, -2C19, -2D6, -3A4, and null) were initially obtained from Nestec Ltd. (Lausanne, Switzerland) under an evaluation license. Human liver samples were obtained, after written and informed consent and ethical approval (Dnr 079-03; Regional Ethics committee in Gothenburg), from the healthy regions of liver lobes from human liver tissue (Hilgendorf et al., 2007) used MVP and PPIA as the most stable housekeeping genes observed in the THLE cell lines. The mRNA concentration was measured using a Nano Drop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE), and RNA purity was assessed from sharpness of ribosomal RNA bands on a 1% agarose gel run in TBE buffer (0.09 M Tris-borate, 0.002 M EDTA, pH 7.8). CDNA was synthesized from qualified mRNA using the Superscript first-Strand Synthesis system, with random hexamer primers as described previously (Hilgendorf et al., 2007). Quantitative PCR was carried out using an ABI PRISM 7900HT Sequence Detection System with custom designed 384-well cards loaded with Assay-on-Demand Gene Expression assays (Applied Biosystems, Foster City, CA), as described by Hayeshi et al. (2008).

**THLE Cell Culture.** THLE cell lines were cultured in flask for maintenance and in 96-well plates before toxicity or metabolism experiments. All cell cultures were maintained at AstraZeneca GSA (Alderley Park, Mere- side, UK), as described previously (Thompson et al., 2012). In this study, cells from the same passage number were analyzed for mRNA expression after culture in 90% confluent flasks and in 96-well plates, to compare the influence of culture conditions.

**RT-PCR Measurement.** The THLE cells were collected 3 days postseeding in 96-well plates, and 90% confluency was attained for the cells in the T175 flasks, by lysis in RNA-STAT60. The cell lysates were collected in 96-well plates, and once 90% confluency was attained for the cells in the culture in 90% confluent flasks and in 96-well plates, to compare the influence of culture conditions. THLE cells (CYP1A2, -2C9, -2C19, -2D6, and null) were initially obtained from Nestec Ltd. (Lausanne, Switzerland) under an evaluation license. Human liver samples were obtained, after written and informed consent and ethical approval (Dnr 079-03; Regional Ethics committee in Gothenburg), from the healthy regions of liver lobes from

**Data Analysis.** Amplification curves were analyzed using SDS2.3 software (Applied Biosystems). The threshold cycle (CT) values obtained for each gene of interest were normalized to the expression level of the housekeeping genes using the formula: Relative mRNA expression level = 2^(-ΔCT), where ΔCT = CT_target gene – CT_housekeeping gene. For “CT housekeeping gene,” the geometric mean of the CT values of the most stable housekeeping genes was used. The most stable housekeeping genes observed in the THLE cell lines were HPRT1, GAPDH, and PPIA, as determined by the Best Keeper algorithm (Pfaffl et al., 2004). Comparisons between mRNA expression in THLE cell lines and in human liver tissue (Hilgendorf et al., 2007) used MVP and PPIA as the most stable reference genes, which were analyzed for all samples.

![Heat map overview of mRNA expression profiles of 96 ADME genes of a selection of liver-typical transporters, phase I and II drug-metabolizing enzymes, and nuclear receptors in THLE cell line genes.](image-url)

**Fig. 1.** Heat map overview of mRNA expression profiles of 96 ADME genes of a selection of liver-typical transporters, phase I and II drug-metabolizing enzymes, and nuclear receptors in THLE cell line genes. Gene names appear in alphabetical order; in parenthesis, the commonly used name of the encoded protein is given. THLE cell lines included in this study are vector-transfected control (Null) and cytochrome P450-transfected cells transfected with either CYP1A2, -2C9, -2C19, -2D6, or -3A4. The color coding indicates relative gene expression levels compared with endogenous reference genes HPRT1, GAPDH, and PPIA: dark green = very high expression level (≥1), light green = high-level (0.3–1), dark blue = moderate level (0.03–0.3), light blue = low expression level (0.03–0.003), white = very low level (<0.003), and a = absent.
Results and Discussion

THLE cell lines expressing a range of individual human P450s are powerful in vitro models for investigating P450-mediated drug metabolism and liver toxicity. However, to our knowledge, the present study is the first that has explored expression of a broad range of hepatic genes in five different P450-transfected THLE cell lines and in THLE-null cells.

We analyzed gene expression of 96 typical ADME genes, which comprised 35 drug-metabolizing enzymes (13 P450 enzymes, two flavin monoxygenases, 20 conjugating enzymes), 43 transporter genes [14 ABC transporters, 29 solute carriers (SLC)], and 12 nuclear hormone receptors alongside seven endogenous controls. An overview of the obtained mRNA expression profiles in the THLE cell lines can be seen in Fig. 1. Our results clearly demonstrated that many human P450 mRNAs were absent or expressed at only very low levels in THLE-null cells and that no significant changes of these low levels of expression occurred after transfection, apart from markedly elevated levels of expression of the transfected P450 genes. These data are in good agreement with previous results obtained by Antolinolobo et al. (2011) and Pfeifer et al. (1993). In addition, our investigation has provided evidence of a low overall expression of many other hepatic ADME genes in the cell lines.

The low expression of many endogenous drug-metabolizing enzymes makes the THLE a cell line suitable for transfection with specific drug-metabolizing enzymes, allowing the generation of in vitro tools to explore the specific enzymes catalyzing a wide variety of metabolic reactions. In THLE-P450 cell lines transfected with P450s 1A2, 2C9, 3A4, and 2D6, high expression levels of the corresponding P450s were evident. In contrast to the THLE cell lines, high mRNA expression levels of P450 enzymes, as well as high levels of 36 additional commonly studied liver genes were detected in cryopreserved human hepatocytes (Fig. 2). Some examples of concordance between mRNA levels and protein expression can be found in the literature (Behrens et al., 2004; Taipalensuu et al., 2004). In this study, functional enzyme activity data for two transfected P450 enzymes (CYP2C9 and CYP3A4) were compared directly to the gene expression levels in the same cell lines. The fold differences in enzyme activity that we observed correlated well with the fold differences in mRNA expression (Fig. 3). Thus, for these two P450 genes, there was a direct relationship between mRNA level and observed enzyme functionality. Although this result cannot be generalized to all genes, it provides reassurance that quantitative mRNA analysis provides a suitable method for profiling a large number of genes of interest. Compared with the pooled human hepatocytes in suspension (CYP3A4 and CYP2C9 activity, 263 and 44 pmol min⁻¹ mg protein⁻¹, respectively), the CYP3A4 activity (8 pmol min⁻¹ mg protein⁻¹) in the THLE-3A4 cell line was lower, whereas the CYP2C9 activity (40 pmol min⁻¹ mg protein⁻¹) in the THLE-2C9 cell line was in the same range. No CYP3A4 or CYP2C9 activity was detected in the THLE-NULL cell line.

As shown in Fig. 1, many of the analyzed genes in this study fell in the absent or low expression level categories in all THLE cell lines. This may be partially due to the embryonic origin of the THLE cells and is consistent with the absence of expression of many key regul-
lating genes such as PXR, CAR, or FXR, which are involved in regulation of important enzymes and transporters (Stahl et al., 2008). In this context, the absence of FXR is consistent with absence of expression of the regulated genes CYP7A1, NTCP, BSEP, OST (Cai and Boyer, 2006). In contrast, moderate levels of expression of AhR and low levels of LXR, RXR, and PPAR gene expression were evident in all cell lines. The abundance of LXR-β and PPAR-γ mRNA was accompanied by the expression of ABCA1. This may be due to the fact that LXR-β is involved in both the transcriptional and posttranscriptional regulation of the ABCA1 transporter (Hozoji et al., 2008). Regarding phase II enzymes, overall the observed expression levels in THLE cell lines fell in the low or absent categories. As an exception, our results showed moderate expression of SULT1A3/4, in contrast to SULT1A1 and SULT2A1, which were absent from all THLE samples. These genes are present at high levels in adult liver (Riches et al., 2009a,b). SULT enzyme activity in THLE cells has not been described earlier, and the potential role of SULT1A3/4 in putative sulfation capacity in THLE cells as pathway for detoxification of xenobiotics has not been investigated. No mRNA expression of glutathione-S-transferase (GST) isoforms GSTA1, GSTA2, and GSTM was detected in the THLE cell line samples, in contrast to the high expression levels of GSTA1 and GSTA2 observed in human hepatocytes and the previously described presence of the GSTP1 isoform in THLE cells (Pfeifer et al., 1993).

The results from our transporter gene expression studies demonstrated the absence or low levels of expression of genes encoding many liver-specific transporter genes (Mizuno et al., 2003), such as OATP1B1 (SLCO1B1) and OCT1 (SLC22A1) in the THLE cell lines. The most highly expressed transporter mRNAs in the THLE cell lines encoded ABCC1, ABCC3, ABCC4, ABCC5, and ABCA1, which each exhibited moderate to high expression levels. No differences were observed between transporter gene expression in THLE-null cells and THLE-P450 cells. Therefore, P450 gene transfection did not exert differential effects on the expression of ABC or SLC transporters. Consequently, in studies that compare toxic responses between different THLE cell lines, no confounding differences caused by effects other than the P450 transfection should be observed.

In conclusion, this investigation shows low expression level or absence of expression of most analyzed genes in the THLE cell lines, with the exception of housekeeping genes and the individual transfectected human P450s. Our results complement previous studies that have demonstrated the value of THLE-P450 cell lines for investigation of the role of P450-dependent metabolism in DILI (Dambach et al., 2004; Benbow et al., 2010; Foster et al., 2010; Greer et al., 2010; Thompson et al., 2012). Therefore, THLE-P450 cell lines provide a useful alternative to isolated human hepatocytes for evaluation of the role of P450-mediated metabolism in formation of toxic metabolites (Greer et al., 2010; Thompson et al., 2011, 2012). However, because overall the THLE cell lines exhibit much lower levels of expression of many genes than human liver, this cell model has key limitations that need to be considered when interpreting data. Low levels of transporter expression in THLE cells may limit entry of substances into the cells, whereas the absence of relevant efflux transporters may lead to metabolites accumulating in the cells in a manner that is not reflective of the in vivo situation (Park et al., 2011; Thompson et al., 2011). In contrast, the low levels of endogenous expression of genes encoding P450s and many other ADME enzymes in THLE cells make them potentially well suited for transfection with many different enzymes, to generate novel in vitro tools enhancing understanding of the role of metabolism in initiation of or protection against drug hepatotoxicity.

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References

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