



Evaluation of a Single-well Cytochrome CYP_{3A4/5}, 1A2 and 2B6 Induction Assay Using Hepatocytes Derived from HepaRG™ Cell Line

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Introduction

- Induction of drug-metabolizing enzymes can represent a major hurdle in drug development.
- Many drugs are enzyme inducers, causing time-dependent changes in pharmacokinetics of drugs metabolized by the induced pathways.
- Enzyme induction may be species-specific; therefore cryopreserved human hepatocytes are commonly used to evaluate potential for enzyme induction.
- A cell line (HepaRG™) was shown to express PXR, CAR and AhR nuclear receptors, and was shown to be inducible with known CYP inducers.

Objectives

- To evaluate and implement a simple, data-rich assay for predicting potential CYP₄₅₀ induction in humans using the HepaRG™ cell line.
- To demonstrate reproducibility of the assay using CYP isozyme-specific inducers
- To compare the extent of induction, based on CYP activity and mRNA quantitation.

Methods

- Cryopreserved HepaRG™ cells, Williams Medium E (WEM), Collagen I Coated 96-well Plates, media and supplements were each obtained from Life Technologies.
- After 72 hours of exposure to inducers, hepatocytes were incubated with three probe substrates [testosterone 200 μM (3A4), bupropion 500 μM (2B6) and phenacetin 100 μM (1A2)]. Formation of 6β-hydroxy testosterone, 3-hydroxy bupropion and acetaminophen at 90 minutes were evaluated, as analyzed by LC/MS/MS
- The mRNA expression of genes of interest was determined with Panomics 2.0 QuantiGene Plex technology (Panomics/Affymetrix, Fremont, CA), following the manufacturer's protocol.
- Drug metabolizing enzymes CYP_{3A4}, CYP_{2B6}, CYP_{1A2} and the "housekeeping gene" were used in this evaluation. Other mRNA data were also collected. The mRNA levels for target genes were normalized to levels of the housekeeping gene, PPIB, based on its similar expression level compared to the P₄₅₀ enzymes monitored.

Conclusions

- By using the HepaRG™ cell line, a high content assay, providing comparable results reproducibly using standards on the same plate was developed. In a single well, the induction of activity and mRNA for the three isoforms 3A4, 2B6 and 1A2 (figures 1, 2 and 3) can be evaluated.
- Correlation between the fold induction in activity and mRNA expression was obtained for positive controls for the 3A4 (rifampicin fold induction of expression/fold induction of activity=1.93), and the 2B6 isoforms (phenobarbital fold expression/fold induction=2.68).
- In the 1A2 positive control there was a larger change in expression as compared to activity for a ratio across experiments of 39.3 of expression to activity in the omeprazole induced samples (figures 2 and 3)
- Mixing the probe substrates in the activity assay, especially when phenacetin is mixed with testosterone, causes an increase in product formation of acetaminophen in rifampicin and phenobarbital induced cells (figures 4 and 6). No signal for 1A2 mRNA induction was detected in these wells (figure 5). These observations are not attributable to induction of CYP_{1A2} activity.

Results

Figure 1. Time-linear formation of CYP_{2B6}, CYP_{3A4} and CYP_{1A2} specific metabolites in induced HepaRG™ cells

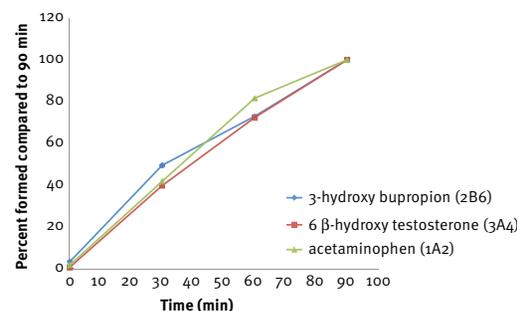


Figure 2. Induced cells show an increase in metabolite formation compared to control (n=3)

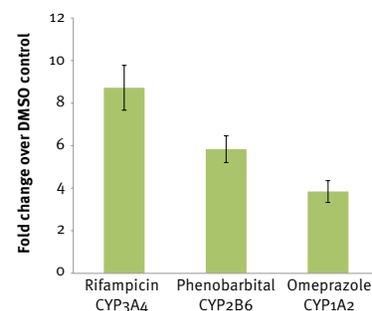


Figure 3. Induced cells show an increase in mRNA expression compared to control (n=3)

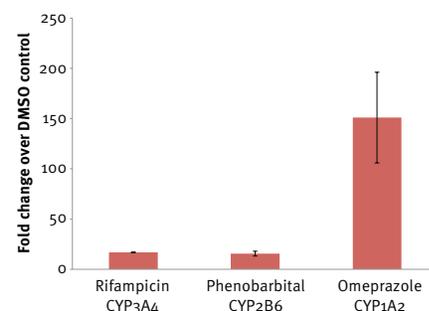


Figure 4. Unexpected time-linear increase in CYP_{1A2} mediated acetaminophen formation by phenobarbital and rifampicin

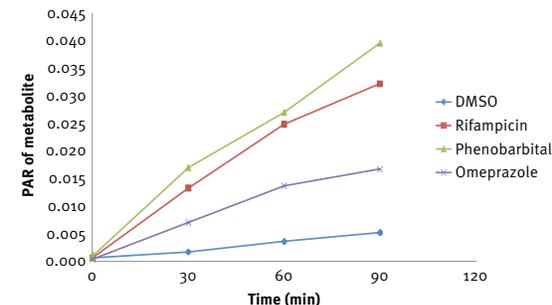


Figure 5. The increase in acetaminophen formation was not accompanied by an mRNA expression increase in the rifampicin and phenobarbital treated cells

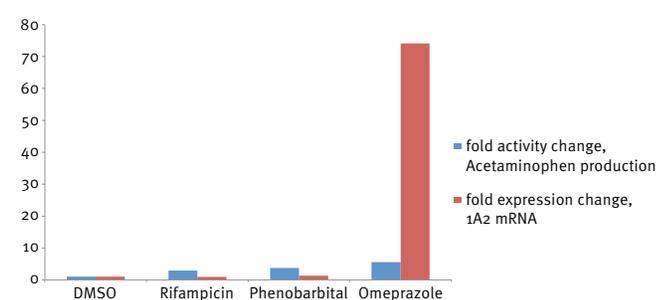
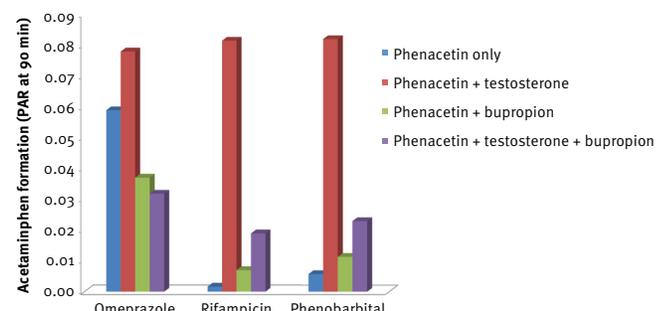


Figure 6. Effect of mixing CYP₄₅₀ substrates on acetaminophen formation from phenacetin in hepatocytes treated with omeprazole, rifampicin or phenobarbital



Future Work

- Evaluate cryopreserved human hepatocytes to check if the mixed substrate effect of testosterone potentiation of acetaminophen production from phenacetin O de-ethylation is present in rifampicin and phenobarbital induced cells.
- Replace testosterone with midazolam in probe substrate assay to evaluate the mixed substrate effect.
- mRNA quantitation can be used to potentially evaluate proteins that do not have defined probe substrate assays. CYP_{1B1}, ABCB₁, ABCG₂ and SLC_{22A1} were measured for mRNA in this experiment (data not shown).
- Evaluate a similar induction assay in co-cultured animal cells that can be connected to in vivo multiple dosed studies in that same species. This could allow the prediction of time dependent changes in pharmacokinetics in animal studies.

Bibliography

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