

Characterizing the induction potential of major cytochrome P450 genes in a novel, all human cell-based hepatocyte tri-culture system



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Introduction

During drug development, novel chemical compounds are assessed for the potential to induce expression of major cytochrome P450 (CYP) enzymes in vitro, typically in sandwich cultured primary human hepatocytes (PHH). While PHH, in combination with regulatory guidelines, can often reasonably predict clinical induction risk, it is still not well understood how newer, more advanced hepatocyte models perform when assessing CYP induction. In this study, a novel, all human cell-based hepatocyte tri-culture system (TCS) was assessed for both the basal expression levels of major CYPs and nuclear receptors, as well as induced levels of CYPs in response to treatment with prototypical inducers. These results define fundamental baseline data for future use of the TCS in in vitro induction studies and provide insight into where the TCS may be able to overcome challenges observed with PHH, such as induction of CYP2C enzymes.

Methods

Evaluation of basal expression of genes over a 4-week culture period

1. PHH and feeder cells were plated on 24-well collagen-coated plates to establish the TCS
2. At specified time points over the course of 4 weeks, wells were treated with TRIzol reagent to lyse cells
3. RNA was isolated using chloroform extraction, and cDNA was generated using the SuperScript IV VIL0 Kit
4. Gene expression was evaluated using a TaqMan array card assay on a QuantStudio 7 Real-Time PCR System. Fold induction was calculated using the $\Delta\Delta C_t$ method compared to Day 1 expression.

Evaluation of CYP expression in response to treatment with prototypical inducers

1. PHH and feeder cells were plated on a 96-well collagen-coated plate to establish the TCS
2. On Day 10 of culture, media containing varying concentrations of prototypical inducers (0.1% DMSO) was added to wells (Table 1)
3. Cells were exposed to inducer for 48 h, with the addition of fresh media and inducer after the first 24 h
4. After 48 h, media was removed, and RNA was collected from each well using the mRNA Catcher PLUS Purification Kit
5. cDNA was generated using the SuperScript IV VIL0 Kit, and gene expression evaluated using TaqMan probes assays on a QuantStudio 7 Real-Time PCR System. Fold induction was calculated using the $\Delta\Delta C_t$ method compared to vehicle controls.
6. E_{max} and EC_{50} values were determined by fitting the data to a sigmoidal model using GraphPad Prism

Table 1. List of CYPs evaluated for induction potential in response to treatment with specified prototypical inducer

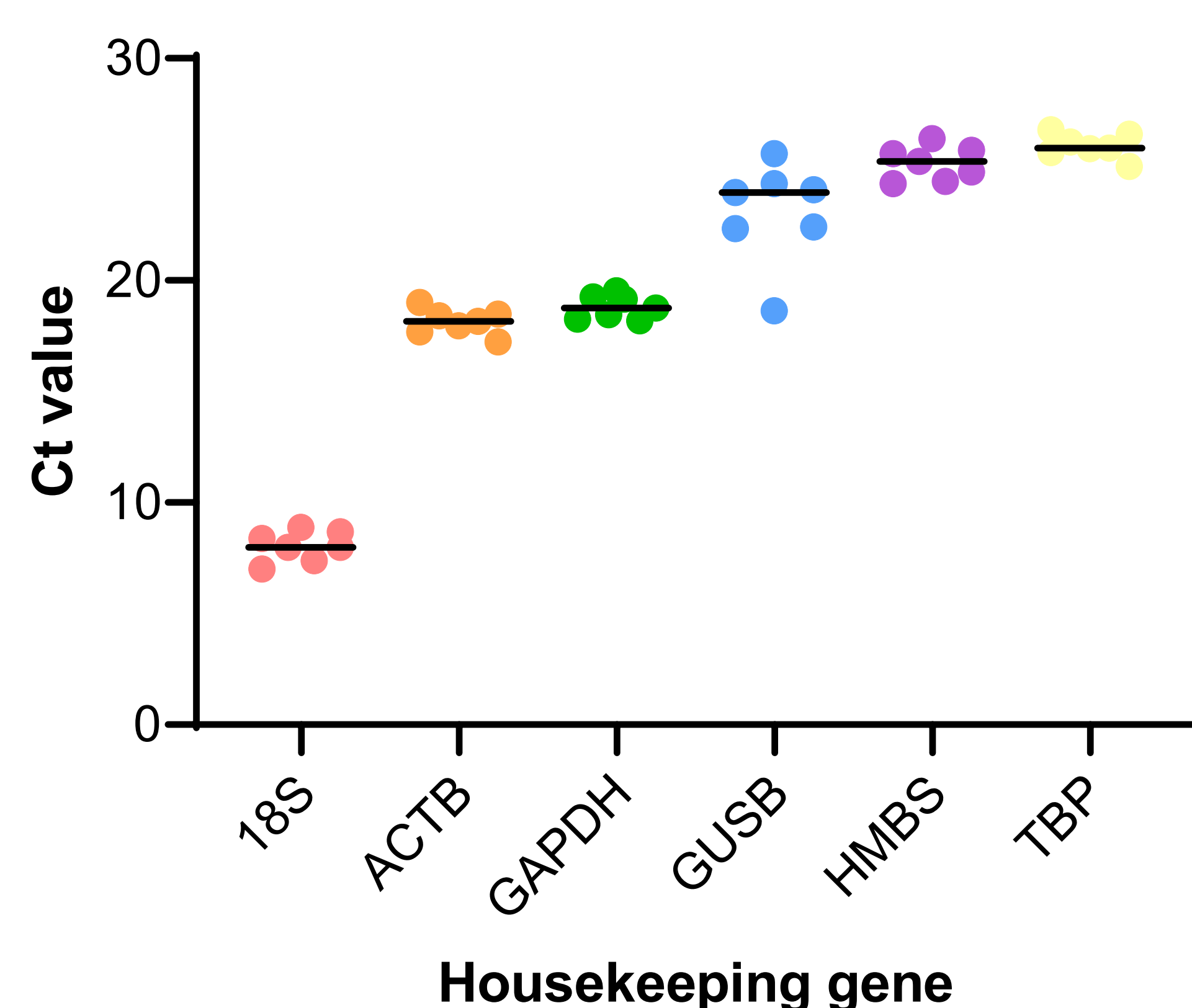
| CYP isoform | Nuclear receptor | Prototypical inducer | Concentration range tested |
|-------------|-------------------------------|----------------------|----------------------------|
| CYP1A1 | Aryl hydrocarbon (AhR) | Omeprazole | 0 – 100 μ M |
| CYP1A2 | | | |
| CYP2B6 | Constitutive androstane (CAR) | CITCO | 0 – 0.5 μ M |
| CYP3A4 | Pregnane X (PXR) | Rifampicin | 0 – 20 μ M |
| CYP2C8 | | | |
| CYP2C9 | | | |
| CYP2C19 | | | |

Table 2. Hepatocyte donor (1921756) information

| Age | Sex | Race | Tobacco use | Alcohol use | Drug use | Medications | Cause of death | Genetic polymorphisms |
|-----|------|-----------|-----------------|------------------|----------|-------------|----------------|-----------------------------|
| 56 | Male | Caucasian | Yes, 1 pack/day | Yes, 6 beers/day | N/A | N/A | CVA/stroke | Wildtype for all major CYPs |

Results

Determination of appropriate housekeeping genes to utilize for studies in the TCS



Descriptive statistics

| Gene | Mean Ct | SD | Range (\pm) |
|--------------|-------------|------------|-----------------|
| 18S | 8.05 | 0.68 | 1.88 |
| ACTB | 18.1 | 0.6 | 1.78 |
| GAPDH | 18.8 | 0.5 | 1.36 |
| GUSB | 23.1 | 2.3 | 7.08 |
| HMBS | 25.3 | 0.8 | 2.04 |
| TBP | 26.1 | 0.5 | 1.65 |

Figure 1. Evaluation of the expression of various housekeeping genes over 4 weeks in culture. Each circle represents gene expression on either Day 1, 4, 7, 12, 17, 23, or 30. GAPDH was selected as the ideal housekeeping gene due to consistency in Ct values over the course of the incubation.

Results

Basal expression of CYPs and nuclear receptors in the TCS over 4 weeks in culture

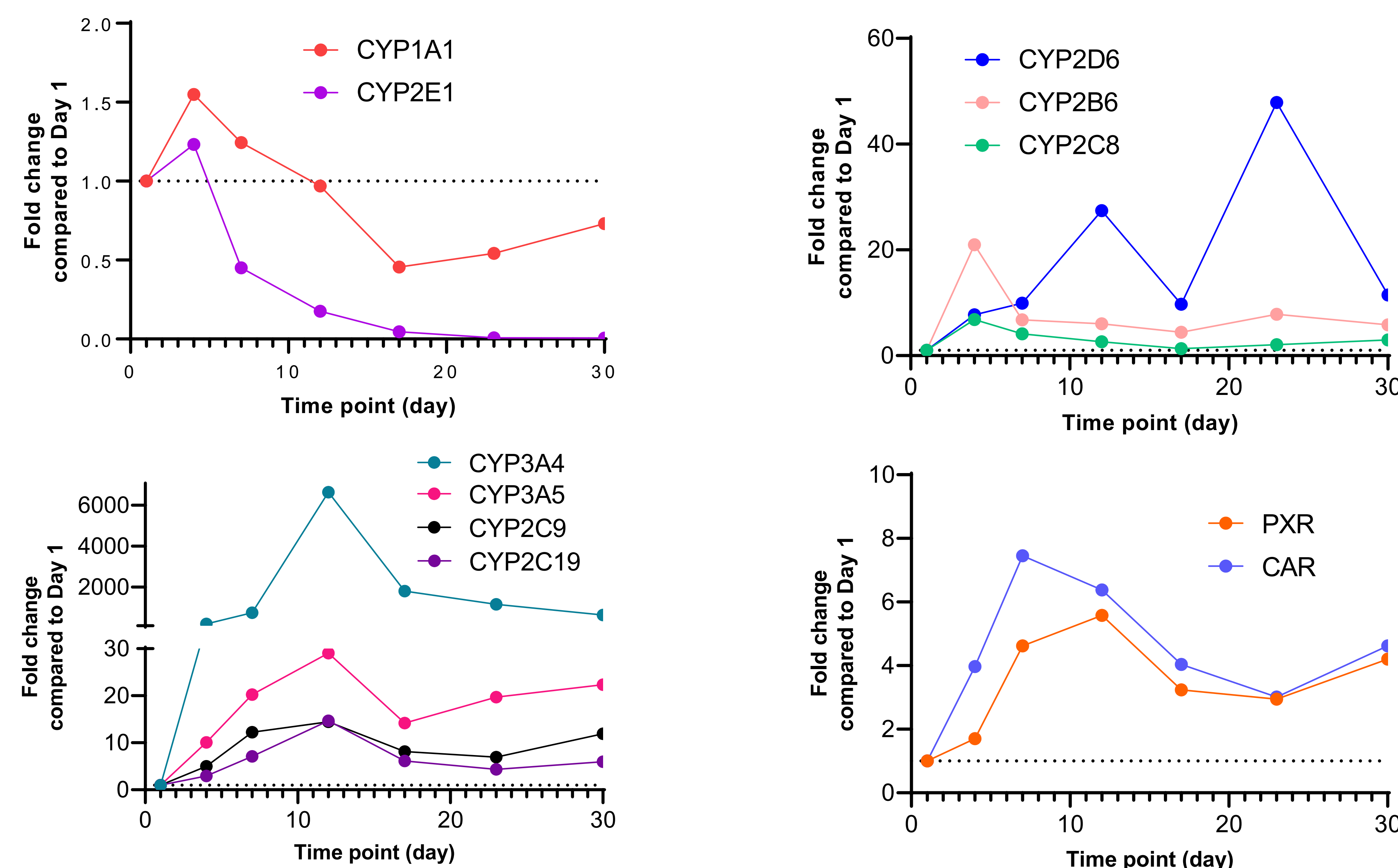
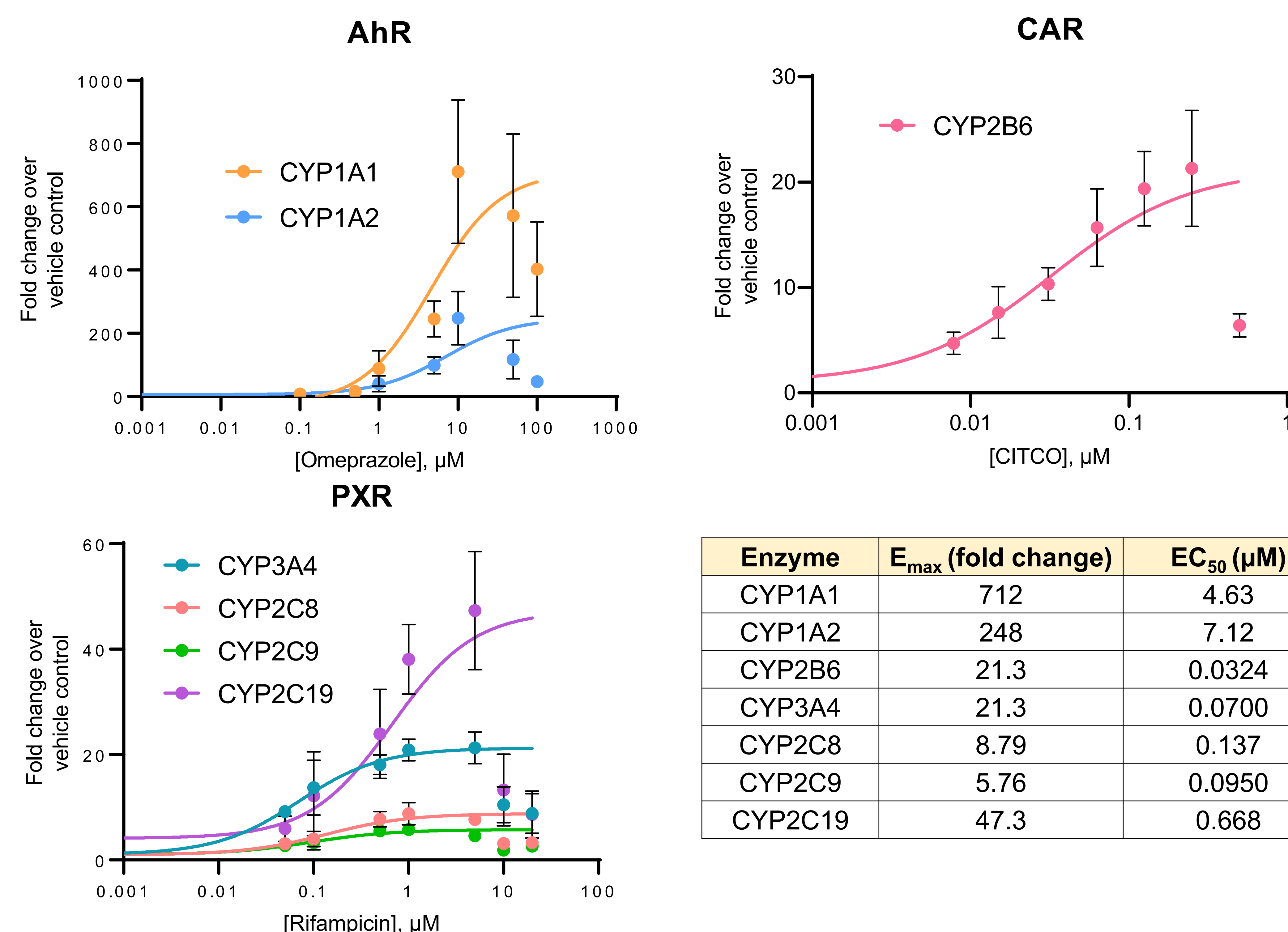


Figure 2. Gene expression of each CYP and nuclear receptor was determined in the TCS on different days over a 4-week culture period under basal conditions. Expression on each day is represented as fold change in comparison to expression levels on Day 1. As most genes reach their highest expression levels between Days 7 – 12, the subsequent induction study in the TCS was initiated on Day 10.

Induction potential of major CYPs following exposure to prototypical inducers for 48 h



| Enzyme | E_{max} (fold change) | EC_{50} (μ M) |
|---------|-------------------------|----------------------|
| CYP1A1 | 712 | 4.63 |
| CYP1A2 | 248 | 7.12 |
| CYP2B6 | 21.3 | 0.0324 |
| CYP3A4 | 21.3 | 0.0700 |
| CYP2C8 | 8.79 | 0.137 |
| CYP2C9 | 5.76 | 0.0950 |
| CYP2C19 | 47.3 | 0.668 |

Figure 3. Gene expression of each CYP was determined in the TCS following treatment with a range of concentrations of various prototypical inducers for 48 h. Expression is represented as fold change in comparison to vehicle control (n = 4), and E_{max} and EC_{50} values for each enzyme were determined by constraining curves to the highest fold change value observed.

Conclusions

- There is a substantial surge in the expression of CYP enzymes (except for CYP1A1 and CYP2E1) and nuclear receptors up to 4-12 days after establishing the TCS
- The expression of most genes tested reach maximal levels in the TCS between Days 7 – 12
- All major CYP enzymes are highly inducible in the TCS in response to prototypical inducers, including the CYP2C enzymes
- Induction potential was observed to decrease at the highest levels of inducers tested for all major CYPs