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Effect of Fifteen CYP3A4 *in vitro* Inducers on the Induction of CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP3A5 in Plated Human Hepatocytes: A Trend Analysis

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Abstract

We previously reported that calibration curve-based approaches generated from CYP3A4 mRNA induction data in human hepatocytes can be used to predict CYP3A4 induction in humans. Here, the same set of samples was analyzed to evaluate induction of CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP3A5 mRNAs. Hepatocytes from three lots were treated with a set of 15 CYP3A4 *in vitro* inducers for 2 days, typically over 8 concentration points to enable calculation of EC_{50} and E_{max}. After treatment, total RNA was isolated and CYP mRNA expression was determined by real-time RT-PCR analysis. Based on E_{max} values, the overall rank order of induction response was 2B6>3A4>2C8>3A5>2C9>2C19, which held for all 3 donors. Rank order and absolute differences often varied by compounds. For example, rifampicin (RIF) induced CYP3A4 by a mean of 10-fold, but was essentially unable to induce CYP3A5. In contrast, phenobarbital (PB) induced CYP3A4 by 7.2-fold and CYP3A5 by 4.9-fold. Nifedipine induced CYP3A4 by 5.6-fold, and induced CYP2C9 by 3.9-fold, whereas RIF and PB induced CYP2C9 by ≤ 2.5-fold. Interestingly, EC_{50} values for CYP3A5 mRNA induction were generally much higher than CYP3A4 (5.5 ± 6.1 fold for 14 compounds that induced CYP3A5). In contrast, EC_{50} values for CYP2B6, CYP2C8 and CYP2C9 were modestly higher (1.7 ± 1.3 to 2.9 ± 4.4-fold greater). Our results demonstrate that induction profiles of CYP2B, 2C and 3A enzymes, that are mediated largely or in part by PXR, can vary substantially by compound and the response is not simply a scaled value of the CYP3A4 induction response. Finally, these results underscore that induction response of certain enzymes not typically considered (e.g. CYP2C8 and CYP3A5) in drug development can be significant.

Introduction

Efficacy loss and altered pharmacokinetics of co-medications due to cytochrome P450 (CYP) induction is a significant concern during drug development. CYP3A4 is highly inducible and is involved in the biotransformation of about half of all drugs that undergo oxidative metabolism. It is well-established that CYP3A4 inducers also induce CYP2B6 and CYP2Cs via overlap in activation and cross talk between nuclear receptors such as pregnane X receptor (PXR) and constitutive androstane receptor (CAR) (1). Therefore, the FDA recommends evaluation of CYP2C enzyme induction should CYP3A induction be observed *in vitro* (2). We previously reported that calibration curve-based approaches generated from CYP3A4 mRNA induction data in human hepatocytes can be used to predict CYP3A4 induction in humans (3). Here, the same set of samples treated with known CYP3A4 inducers was analyzed to evaluate induction of CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP3A5 mRNA.

Materials and Methods

Materials. All model test drugs were obtained from Sigma-Aldrich. Cryopreserved human hepatocytes (Lots 295, 312, and 318) were obtained from Corning Life Sciences (Tewksbury, MA).

Hepatocyte plating and treatment. Cryopreserved hepatocytes were thawed using Corning® Gentest™ High Viability CryoHepatocyte Recovery Kit and plated in Corning Collagen I-coated 96-well plates. Cell cultures were maintained in Corning Hepatocyte defined medium supplemented with glutamine, gentamicin and fungizone, overnight prior to treatment with model drugs at eight concentrations for 48 h (Table 1). After treatment, total RNA was isolated using Qiagen RNeasy® 96 Kit. The mRNA expression for each isoform was determined under the two-step protocol using a model 7300 or ViiA 7 Real-Time PCR System (Taqman®, Applied Biosystems).

Data analysis. Mean fold induction over solvent vehicle control for mRNA expression from triplicate treatment samples was used to calculate EC_{50} and E_{max}. The calculation was conducted with XLfit™ (IDBS) curve-fitting software using a 4 Parameter Logistic Model, no. 205. Acceptance criteria for the fits are described previously (3). When a discernible plateau was not evident or no induction response was found, the maximal observed fold over control was used for analysis. Inducibility of CYP isoforms was determined by frequency of a >2 fold induction of each enzyme for these compounds in the three hepatocyte lots. E_{max} distribution, and a paired EC_{50} graph between CYP3A4 vs. CYP3A5 or CYP3A4 vs. CYP2C8 of all compounds were used to visualize distinct inducibility patterns of CYP isoforms where. The EC_{50} values were converted to molar units prior to log transformation comparison analysis of EC_{50}.

Table 1. EC_{50} of CYP mRNA Induction by Model Test Drugs

Test Drug	Test concentration range (µM)	CYP3A4		CYP3A5		CYP2B6		CYP2C8		CYP2C9	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Rifampicin	0.01-50	0.65	0.67	nd	-	0.60	0.28	0.21	-*	0.59	-*
Phenytoin	0.23-500	12	2.4	35	-**	3.2	1.9	8.7	7.1	9.4	-**
Carbamazepine	0.23-500	16	11	142	51	22	9.7	22.0	18	30	-*
Phenobarbital	0.91-2000	153	78	592	211	247	157	205	43	228	11
Troglitazone	0.03-20	3.7	2.4	17	12	15	7.5	12	4.6	12	-*
Terbinafine	0.05-100	4.5	3.6	16	8.6	6.0	3.5	8.1	3.7	5.3	5.2
Pleconaril	0.05-100	4.2	1.9	12	2.7	1.1	0.27	2.2	0.25	nd	-
Dexamethasone	0.11-250	26	4.6	29	4.9	34	1.6	34	2.8	55	21
Sulfinpyrazone	0.09-200	16	10	30	8.0	34	9.4	29	7.5	31	7.2
Probenecid	0.05-300	80	42	50	33	64	49	56	19	63	-*
Nifedipine	0.05-100	9.1	3.5	23	17	8.8	1.5	22	15	6.8	5.9
Pioglitazone	0.05-100	3.4	0.50	3.4	-*	3.8	0.21	2.2	1.0	2.8	-*
Rosiglitazone	0.05-100	8.8	1.1	28	12	16	6.0	11	2.1	9.2	-*
Omeprazole	0.05-100	6.4	-*	26	13	20	13	nd	-	nd	-
Clotrimazole	0.005-10	3.3	0.75	4.7	3.0	3.4	0.64	4.1	0.50	4.0	3.4

Data are the mean ± standard deviation from three hepatocyte lots except where indicated [* n=2 lots ** n=1 lot; nd=not determined (EC_{50} calculation was not achievable for all lots)]

Figure 1. Paired-data Analysis of EC_{50} Values for CYP3A4 vs CYP3A5, and CYP3A4 vs CYP2C8

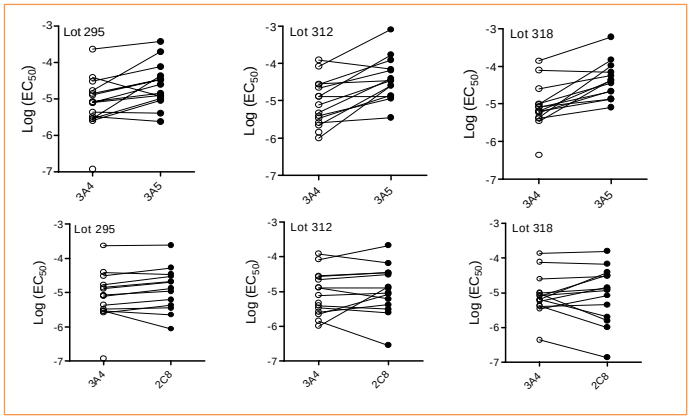
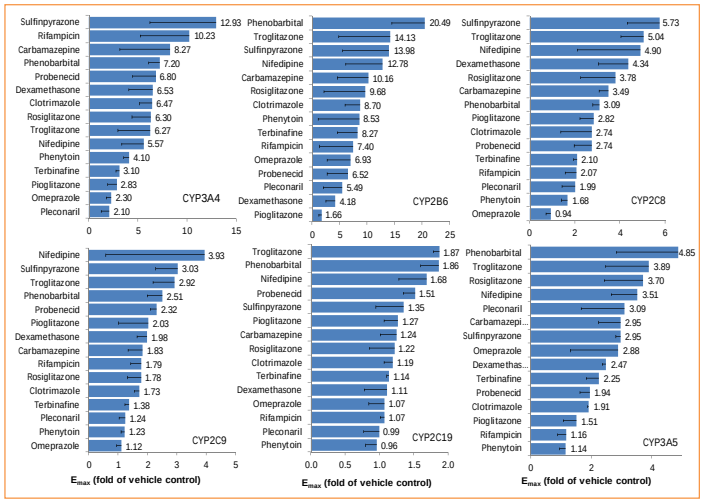
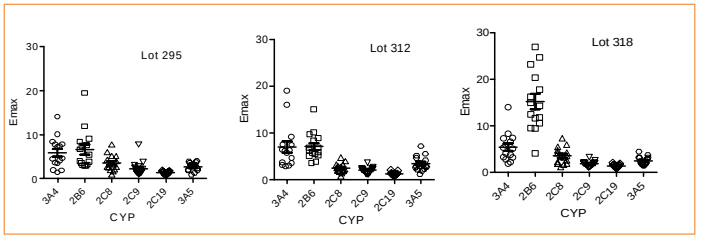


Figure 2. E_{max} of mRNA Induction by Model Test Drug in Three Hepatocyte Lots.



Data are the mean ± standard deviation from three hepatocyte lots.

Figure 3. Distribution Analysis of E_{max}



Notable Trends and Observations

- EC_{50} values for CYP3A5 mRNA induction were on average much higher than CYP3A4 (5.5-fold) (Table 1 and Fig. 1). By comparison, EC_{50} values for CYP2B6, CYP2C8 (Fig. 1) and CYP2C9 were on average (1.7- to 2.9-fold higher).
- The overall rank order of maximum fold-induction response was 2B6>3A4>2C8>3A5>2C9>2C19, a pattern which held for each of the three hepatocyte lots (Fig. 3), despite some notable interindividual differences (e.g. CYP2B6 with lot 318)
- Within compounds (Fig. 2), notable exceptions to the rank order included:
  - Pioglitazone: CYP3A4 = 2C8 > 2B6 > others
  - Pleconaril and omeprazole: CYP2B6 > 3A5 > 3A4 > others
  - Rifampicin: CYP3A4 > 2B6 > 2C8 > 2C9 > 3A5 (no induction)
  - Phenobarbital: CYP2B6 > 3A4 > 3A5 (4.9-fold) > others
- Troglitazone and sulfinpyrazone gave the highest response for CYP2C8 (5.0 and 5.7-fold, respectively); rifampicin (2.1-fold) and phenobarbital (3.1-fold) were much less. These data have implications for selection of a robust positive control for CYP2C8 induction response.
- Relative to other compounds, rifampicin, exhibited high selectivity for CYP3A4 maximal induction; Based on its EC_{50} value, it was by far the most potent inducer for all CYPs, except for CYP2C19 and CYP3A5 (no induction)

Conclusion

- Our results demonstrate that induction profiles of CYP2B, 2C and 3A enzymes can vary substantially by compound. Therefore, an assumption that induction response may be simply a scaled value of a "sentinel" P450 induction response (such as CYP3A4 for PXR-mediated induction) would appear risky.
- Our results underscore that maximal induction response of certain enzymes not typically considered (e.g. CYP2C8 and CYP3A5) in drug development can be significant.
- The EC_{50} values (which are a measure of induction potency) for CYP3A5 are notably higher than those for CYP3A4.
- These data provide a useful data-mining set. For example, several compounds were identified that are likely more robust positive controls for CYP2Cs and CYP3A5 (e.g. troglitazone for CYP2C8, 2C9 and 3A5) than rifampicin, a compound typically selected for such analyses.

References

(1) Fahmi O et al. (2010) Drug Metabolism and Disposition 38:1605.

(2) FDA guidance for Drug Interaction Studies (2012)

(3) Zhang JG et al (2014) Drug Metabolism and Disposition 42:1379.