THE RELATIONSHIP BETWEEN mRNA AND ACTIVITY FOLLOWING INDUCTION OF CYP3A4 AND CYP2B6 ACROSS A RANGE OF PROTOTYPICAL INDUCERS

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Introduction

- CYP induction can be quantified using a variety of markers including an increase in mRNA expression, an increase in protein expression or an increase in enzyme activity.
- The use of mRNA data (Ind_{max} and $IndC_{50}$) to predict the magnitude of interaction using mechanistic models is recommended as a sensitive marker that is not impaired when a drug exhibits mechanism-based inhibition and induction of the same enzyme simultaneously [1, 2].
- However, any difference between induction efficacy and potency derived from mRNA vs. activity data can impact the predicted magnitude of interaction.

Aims

- The aim of this study was to investigate the relationship between mRNA and activity for both 3A4 and 2B6 within the same hepatocyte donor and experiment.

Methods

- The change in CYP3A4 and CYP2B6 mRNA expression and enzyme activity were assessed in parallel in cryopreserved human hepatocytes from 4 donors.
- Hepatocytes were incubated for 48 hours with varying concentrations of 6 inducers (rifampicin, carbamazepine, phenobarbital, phenytoin, efavirenz and nifedipine) prior to *in situ* assessment of activity (formation of 6β-hydroxytestosterone and hydroxybupropion) and mRNA levels (QuantiGene Plex 2.0 Affymettrix Assay Kit) in the same well as previously described [3].
- Cell toxicity and viability were monitored using LDH leakage and AlamarBlue® assays.
- Data for mRNA and activity were plotted as fold increase over vehicle control vs. the concentration of the inducer and curve fitting carried out to derive the mean Ind_{max} (maximum fold induction, E_{max} + 1) and $IndC_{50}$ (the concentration that yields half of the maximum fold induction).

Results

- For CYP3A4 induction, mRNA data yielded higher efficacy (mean 1.7-fold higher Ind_{max}) and lower potency (mean 2.2-fold higher $IndC_{50}$; Figure 1a).
- For CYP2B6 induction, mRNA data yielded lower efficacy (mean 0.5-fold) and lower potency (higher $IndC_{50}$, mean 3.7-fold; Figure 1b).

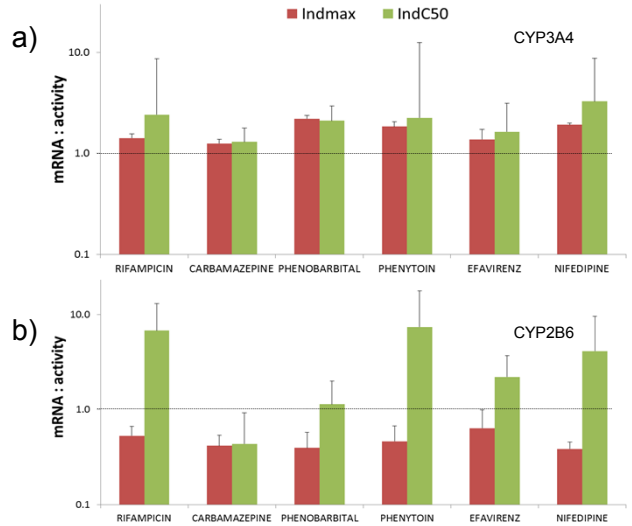

Figure 1 The fold difference in Ind_{max} (red) and $IndC_{50}$ (green) values determined from mRNA and activity data for a) CYP3A4 and b) CYP2B6. Data are plotted as mean +/- SD.

Results Cont.

- When the ratio of Ind_{max} : $IndC_{50}$ for CYP3A4 across compounds was compared in mRNA and activity it was similar (median ratio 0.9, range 0.6-1.4) and correlated (r^2 >0.99; Figure 2a & 3a).
- When the ratio of Ind_{max} : $IndC_{50}$ for CYP2B6 across compounds was compared in mRNA and activity they were lower in mRNA compared to activity (median ratio 0.42, range 0.12-.4.9-fold) and poorly correlated (r^2 = 0.04; Figure 2b &3b).

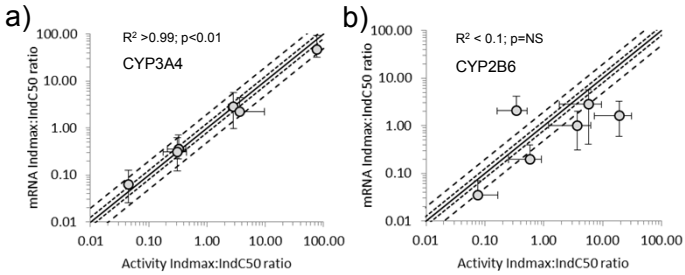

Figure 2 The relationship between the ratio of Ind_{max}:$IndC_{50}$ in mRNA vs. activity for a) CYP3A4 and b) CYP2B6. Data are plotted as mean +/- SD. The lines of unity (solid), 1.8-1.25-fold (dotted) and 0.5-2-fold (dashed) are shown for reference.

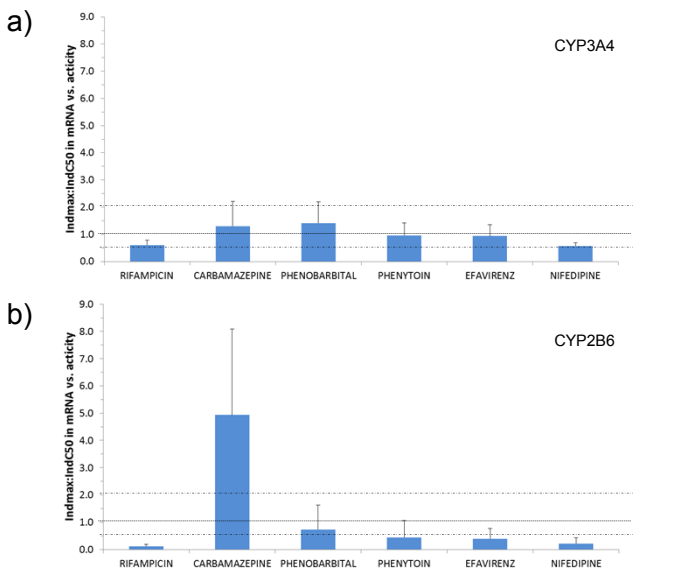

Figure 3 The fold difference in the induction factor (Ind_{max}:$IndC_{50}$) between mRNA and activity for a) CYP3A4 and b) CYP2B6. Data are plotted as mean +/- SD. The line of unity and +/- 2-fold are shown for reference.

Conclusions

- These data indicate that the predicted magnitude of interaction using Ind_{max} and $IndC_{50}$ generated from mRNA or activity data would be similar for CYP3A4 but not for CYP2B6.
- Although a caveat of this work is that a specific CAR activator was not included, it suggests that the relationship between mRNA and activity should be evaluated for each enzyme prior to use in DDI predictions using mechanistic static or dynamic models.
- Future investigation of these relationships across different laboratories would be beneficial to add to our understanding of how best to extrapolate *in vitro* CYP2B6 induction data to *in vivo*.

References

1.FDA (2012) Guidance for Industry: Drug Interactions Studies - Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations, Services USDoHaH ed, pp 1-79, FDA, Silver Spring.

2.Fahmi et al., 2010 Cytochrome P450 3A4 mRNA is a more reliable marker than CYP3A4 activity for detecting pregnane X receptor-activated induction of drug-metabolizing enzymes. Drug Metab Dispos. Sep;38(9):1605-1,

3.Halladay JS et al., 2012 An 'all-inclusive' 96-well cytochrome P450 induction method: measuring enzyme activity, mRNA levels, protein levels, and cytotoxicity from one well using cryopreserved human hepatocytes. J Pharmacol Toxicol Methods 2012 66:270-75.