Correlation Between Cytochrome P450 Inducers and Nuclear Receptor Activation - a Screening Approach

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Abstract # 92

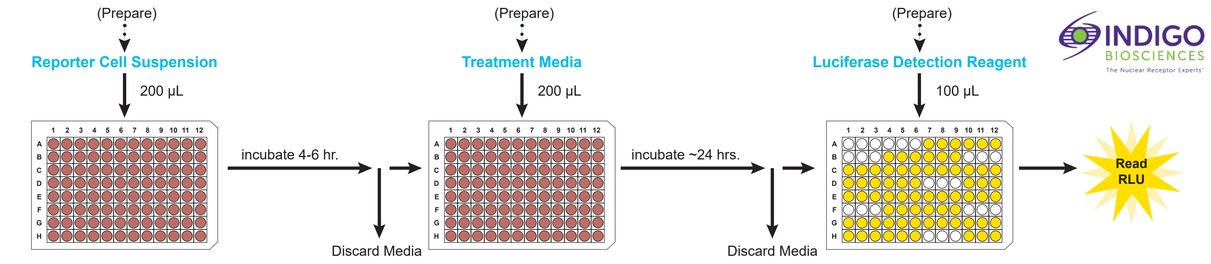
Abstract

Cytochrome P450 (CYP) enzymes play a major role in the metabolism of xenobiotics. According to FDA Guidance (October 2017), sponsors should initiate *in vitro* metabolic studies before first-in-human studies to influence the design of clinical PK studies assessing the potential for interactions between CYPs and investigational drugs / new molecular entities. It is recommended to the evaluate the potential to induce CYP1A2, CYP2B6, and CYP3A4/5, with only further investigation in the potential to induce CYP2C8, CYP2C9 and CYP2C19 should there be induction of CYP3A4/5 as induction is mediated via activation of the pregnane X receptor (PXR). AhR (Aryl Hydrocarbon Receptor), though not a member of the Nuclear Receptor Family, shares many of the same attributes as PXR and via formation of a heterodimer with ARNT, leads to increased transcription of CYP1A1, CYP1A2, CYP2B1 and UGT1A6, and thus new molecular entities could be investigated as possible CYP1A2 inducers via AhR activation. Constitutive Androstane Receptor (CAR) is a nuclear receptor that activates the transcription of several target genes in CYP mediated metabolism, with isoform 3 predominantly involved in the induction of CYP2B6 and thus new molecular entities could be investigated as possible CYP2B6 inducers via CAR3 activation. Nuclear receptor assays are often done using reporter cell lines in a ready to assay format with data turnaround in 24 hours. In previous work, we evaluated a high throughput format using 24 drugs with known CYP induction properties to validate the induction of CYP1A2, CYP2B6 and CYP3A4 across different donor hepatocyte lots, however, in a screening format, this could lead to a significant amount of cost in procurement of reagents and cells as well as variability in the potential to induce enzymes due to hepatocyte donor expression levels. Using the same series of reference compounds, several which are CYP2C inducers, we correlate the activation of AhR, PXR and CAR3 with the induction of CYP1A2, CYP2B6, and CYP3A4 enzymes and demonstrate the utility of these assays to evaluate induction of CYP enzymes as directed by FDA Guidance.

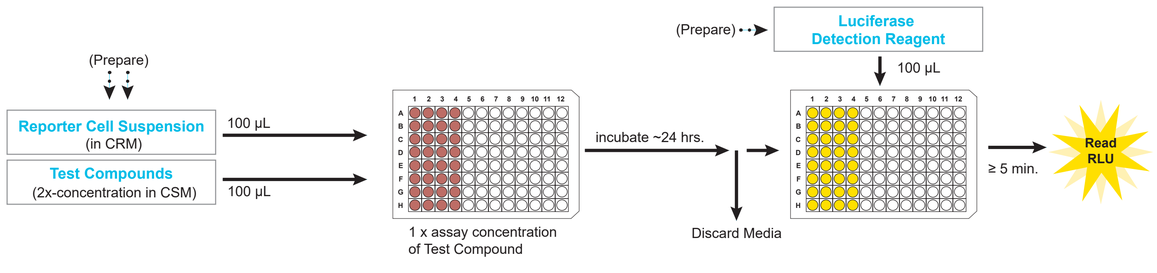
Methods

Cytochrome P450 Induction experiments in primary human hepatocytes were performed as previously described[1]. Nuclear Receptor Activation Kits were procured from Indigo Biosciences (State College, PA) and performed per manufacturer's instruction. Briefly, reporter cells corresponding to Aryl Hydrocarbon Receptor (AhR), Constitutive Androstane Receptor Isoform 3 (CAR3), and Pregnane Xenobiotic Receptor (PXR) were suspended in Cell Recovery Media (CRM, Indigo Biosciences, State College, PA) and allowed to attach to white opaque 96-well plates (Corning Life Sciences, Corning, NY) for 4-6 hours. Compounds were spiked into Compound Screening Media (CSM) and diluted serially for generation of EC_{50} curves. After the cellular attachment period, the CRM was replaced with CSM spiked with compounds. After 24 hours, CSM was decanted and luminescent substrate was added to the plate and allowed to incubate for 5 minutes. The plates were then analyzed on a Synergy HTX (BioTek Instruments, Winooski, VT). Data was exported to Microsoft Excel and regressed using GraphPad Prism Software (GraphPad Software Version 8.0.1, San Diego, CA).

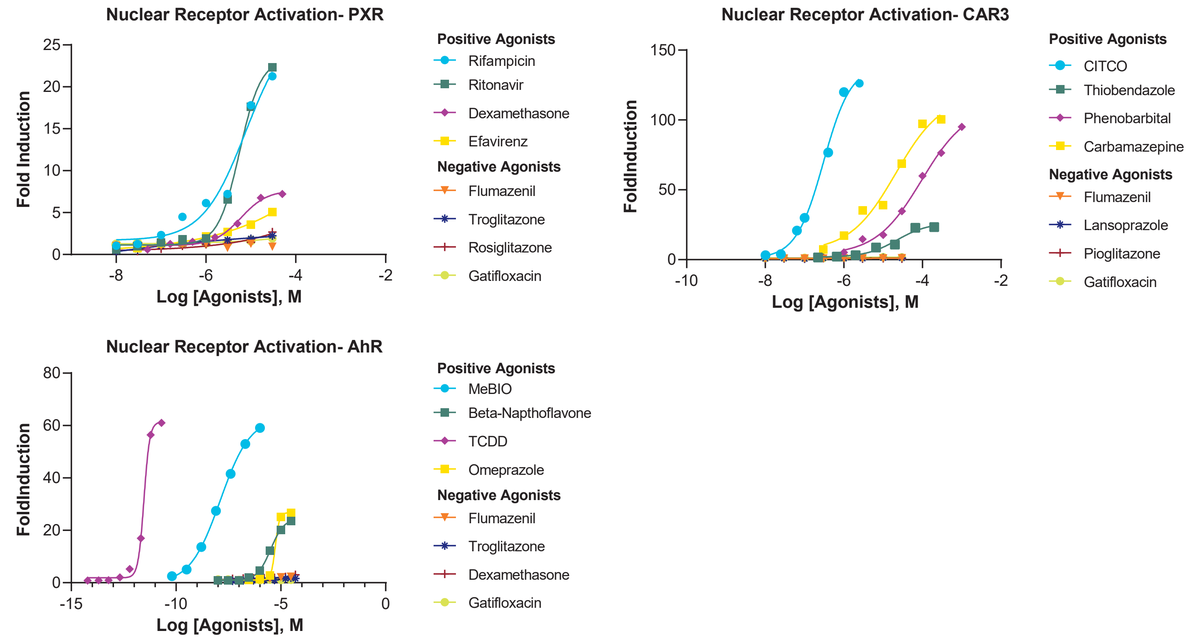
Assay Scheme - AhR and PXR Assays



Assay Scheme - CAR3



Agonist Dose Response Analyses of AhR, PXR and CAR3 and Correlation Between CYP Inducers and Non-Inducers



Compound	Top Concentration in CYP and NR Assays (uM)	AhR Fold Induction	CAR3 Fold Induction	PXR Fold Induction	CYP1A2 Fold Induction	CYP2B6 Fold Induction	CYP3A4 Fold Induction	CYP1A2 Inducer	CYP2B6 Inducer	CYP3A4 Inducer
Ranitdine	30.0	1.1	2.3	2.0	1	1.1	1.1	No	No	No
Ritonavir	30.0	2.1	4.5	22.3	0.9	4.4	29.6	Weak	Weak	Yes
Rosiglitazone	5.0	1.5	37.5	2.7	2.7	2.9	1.5	Yes	Yes	No
TCDD	0.2	61.1	1.0	2.2	60.4	0.7	1	Yes	No	No
Thiabendazole	200.0	1.1	23.3	3.0	10.8	1.6	1	Yes	Yes	No
Topiramate	100.0	1.4	0.2	1.2	0.8	2.8	1	No	No	No
Troglitazone	50.0	1.9	21.9	12.4	3.8	3.3	5	Yes	Yes	Yes
Beta-Napthoflavone	30.0	23.6	1.0	0.9	54.3	1	1	Yes	No	No
Phenobarbital	1000.0	1.3	95.1	5.4	1.5	19.4	84.2	Weak	Yes	Yes
Omeprazole	50.0	17.0	93.8	6.8	90.5	5.7	8.5	Yes	Yes	Yes
Rifampicin	30.0	26.7	7.2	42.3	0.5	9.7	79	Weak	Weak	Yes
Flumazenil	30.0	1.7	0.8	1.0	1.2	1.1	1.1	No	No	No
3-Methylcholanthrene	2.0	36.8	1.0	1.2	36.3	1	1	Yes	No	No
CITCO	10.0	3.6	100.6	3.0	2.7	5.1	11.6	No	Yes	Weak
Dexamethasone	50.0	3.0	0.8	7.2	1.2	3.6	19	No	No	Yes
3,3-diindolylmethane	5.0	45.7	0.8	1.4	36.3	1.6	1	Yes	No	No
Efavirenz	30.0	49.3	38.6	5.1	1	4.8	14.2	No	Yes	Yes
Gatifloxacin	30.0	1.2	0.8	1.0	1	0.9	1.5	No	No	No
Lansoprazole	30.0	7.9	1.2	1.3	15.8	2.9	6.1	Yes	No	No
Carbamazepine	250.0	1.4	10.3	4.3	9.1	13.8	38.3	Weak	Yes	Yes
Montelukast	50.0	2.8	0.6	1.1	2	0.8	1	Yes	No	No
Nevirapine	250.0	1.5	8.8	1.2	3.8	5.1	1	No	Yes	No
Phenytoin	100.0	1.5	15.2	1.2	2.5	9.3	39.9	Yes	Yes	Yes
Pioglitazone	50.0	1.9	1.3	1.0	1.7	1.5	1	Weak	No	No

Figure 1. Agonist Dose Response Analyses of AhR, PXR and CAR3 and Correlation Between Known CYP Inducers and Non-Inducers. Agonist analyses were performed using kit protocols from Indigo Biosciences (State College, PA) with n=2 replicates. MeBio, CITCO and Rifampicin are positive controls provided with AhR, CAR3 and PXR kits respectively. Representative of 4 inducers and 4 non-inducers are shown for AhR (CYP1A2), CAR3 (CYP2B6) and PXR (CYP3A4). Representative EC_{50} curves are shown here. Fold-activation was calculated versus vehicle (0.1% DMSO in Compound Screening Medium). The fold induction was correlated for each Nuclear Receptor against known CYP Induction mRNA and represented as Log Fold Induction-Nuclear Receptor (NR) versus Log Fold Induction-mRNA. against previously validated lots of human hepatocytes (Lot# CDP, BioIVT, Westbury, NY). Good agreement was shown between known CYP inducers and non-inducers for all 24 compunds tested in Nuclear Receptor Activation Assays.

Summary

Nuclear Receptor Activation is critical prior to induction of expression of CYP enzymes. There is good correlation between known CYP Inducers and Nuclear Receptor Activation. Using this screening approach with reporter cell lines, one can evaluate the potential to induce three major CYP Isoforms 1A2, 2B6 and 3A4 by activation of AhR, CAR3, and PXR Respectively. Omeprazole, CITCO, and Rifampicin are the positive controls used in Cyp Induction mRNA assays show good activation in these reporter cell lines, as well as Flumazenil, which is a negative control in Cyp Induction mRNA assays. Nuclear Receptor Activation Assays also provide a lower cost alternative to expensive hepatocyte assays and can be used for both screening prior to confirmation by mRNA/enzyme activity assays, but also can be used to describe the mechanism for induction, necessary in evaluating Drug-Drug Interactions (DDIs), critical for regulatory submission based on recent regulatory guidance.

References

1. In Vitro Metabolism- and Transporter- Mediated Drug-Drug Interactions Studies Guidance for Industry- *FDA*, October 2017
2. Prakash C, *et al*, "Nuclear Receptors in Drug Metabolism, Drug Response and Drug Interactions," *Nucl Receptor Res.* 2015 ; 2: . doi:10.11131/2015/101178.
3. Fahmi OA, *et al*, "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.* 38(9):1605-1611, 2010.