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In vitro **ADME & PK**

Cytochrome P450 Induction

Background Information

'Cultured hepatocytes... are the preferred *in vitro* system for induction (and down-regulation) *in vitro* studies.'

[2]EMA (2012) Guideline on the investigation of drug interactions

- Induction of cytochrome P450 enzymes is associated with an increased prevalence of clinical drug-drug interactions.
- Cyprotex's Cytochrome P450 induction assay identifies the potential of test compounds to induce CYP1A2, CYP2B6 or CYP3A4 in cultured human hepatocytes by evaluating mRNA levels and/or catalytic activity. Assays are designed to meet FDA[1] and EMA[2] guidelines.
- Test drug concentrations should be based on the expected human plasma drug concentrations and dose. Solubility, cytotoxicity and plasma protein binding should also be taken into consideration.
- Cyprotex's Cytochrome P450 induction assay delivers fold-induction data normalised to vehicle control which can be compared to positive control responses. If appropriate, data is fit using non-linear regression analysis to four-parameter sigmoidal equation to produce E_{max} and EC_{50} values.
- The clinical consequences of induction may be therapeutic failure caused by a decreased systemic exposure of the drug itself or a co-administered therapy, or toxicity as a result of increased bioactivation.

Supplementary Assays

Preliminary aqueous solubility assessment

Preliminary cytotoxicity assessment using MTT in primary human hepatocytes or HepaRG™

Measurement of parent drug on final day of dosing

Relative induction score (RIS) analysis

Protocol

Test System
Cryopreserved hepatocytes (3 donors recommended)
HepaRG™ cells are also available on request

Test Article Concentration
6 concentrations (dependent upon unbound C_{max}, dose, solubility and cytotoxicity) plus vehicle control, in triplicate (alternative number of concentrations may be available on request)

CYP Isoforms
CYP1A2, CYP2B6 and CYP3A4
For CYP2C8, CYP2C9 & CYP2C19 please contact directly for information

Controls
Omeprazole (CYP1A2 positive control)
Phenobarbital (CYP2B6 positive control)
Rifampicin (CYP3A4 positive control)
Flumazenil (negative control)

Test Article Requirements
Dependent on top concentration (recommend 0.1% DMSO in incubation)

Exposure Period
72 hr (media changed every 24 hours)

Probe Substrates for Catalytic Activity
Phenacetin (CYP1A2)
Bupropion (CYP2B6)
Midazolam (CYP3A4)

Analysis Method
LC-MS/MS quantification of acetaminophen (CYP1A2), hydroxybupropion (CYP2B6) and 1-hydroxymidazolam (CYP3A4)
qRT-PCR for relative mRNA expression levels (CYP1A2, CYP2B6 and CYP3A4)

Data Delivery
Excel sheet detailing mRNA levels, fold induction relative to vehicle control, concentration of metabolite of probe substrate, E_{max}, EC_{50} and F2 (concentration which leads to a 2-fold increase above E_{min}) if appropriate. Written report available on request

To find out more contact **enquiries@cyprotex.com**

‘The sponsor should evaluate the potential of an investigational drug to induce CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP3A4... If the result from at least one donor exceeds the pre-defined threshold, the sponsor should consider the drug to have induction potential and conduct a follow-up evaluation.[1]’

Figure 1

Induction of CYP1A2 mRNA levels by omeprazole in cryopreserved human hepatocytes.

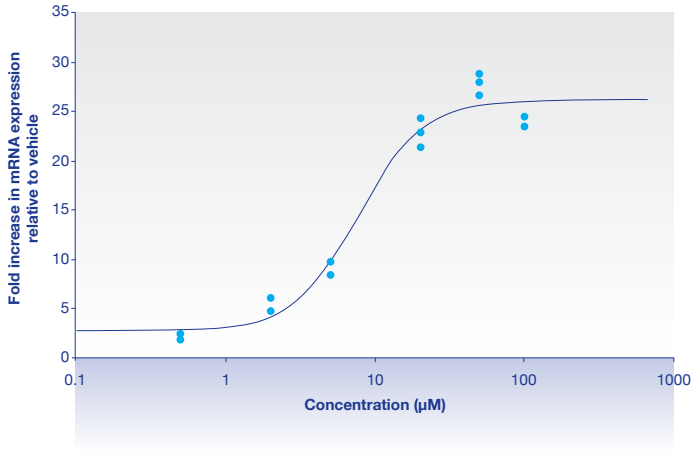


Figure 2

Induction of CYP2B6 mRNA levels by phenobarbital in cryopreserved human hepatocytes.

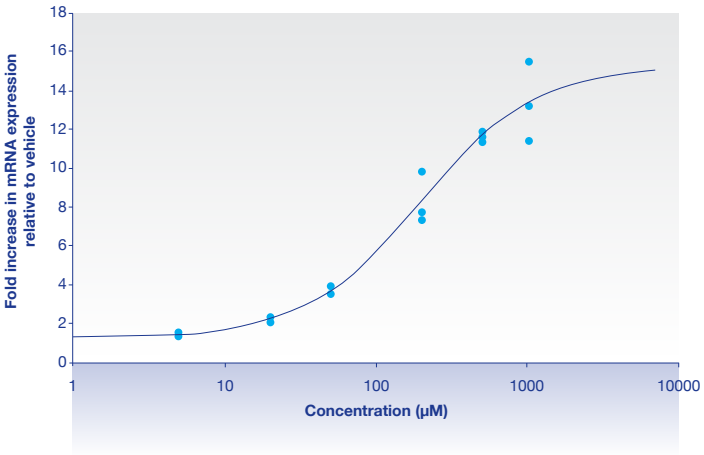
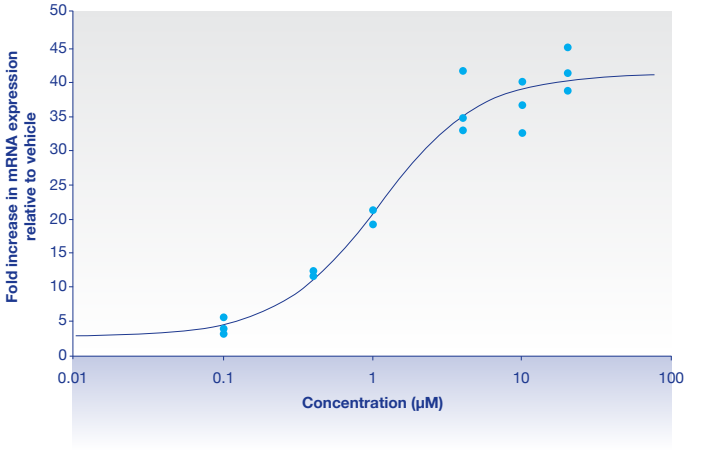


Figure 3

Induction of CYP3A4 mRNA levels by rifampicin in cryopreserved human hepatocytes.



References

[1] FDA (2020) Guidance for industry: drug interaction studies-study design, data analysis, implications for dosing, and labeling recommendations.
[2] EMA (2012) Guideline on the investigation of drug interactions.